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3501

LEIGH SYNDROME

P.M.M. VAN ERVEN

LEIGH SYNDROME

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PROEFSCHRIFT

ter verkrijging van de graad van
Doctor in de Geneeskunde
aan de Katholieke Universiteit te Nijmegen,
op gezag van de Rector Magnificus
Prof.Dr. B.M.F. van Iersel
volgens besluit van het College van Decanen
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Ter herinnering aan mijn vader

voor Silvy, Saskia en Moniek

CONTENTS

PREAMBLE

In recent years, knowledge in the field of medical sciences has been expanding more rapidly than ever before. For centuries medicine was a static discipline, based on the paradigm of humoral pathology. After the opening of the human body in the Renaissance the apodictic concepts of the Antics were gradually abandoned and replaced by insights based on observation and measurable data. The development of the natural sciences gave an impetus to the developing medical science, and medical classification and nosology were based mainly on clinical signs and symptoms, and on pathology.

Modern techniques in electrophysiology, radiology, chemistry, biochemistry, and pathology changed fundamentally our concepts of etiology and pathophysiology. Especially, immunology, molecular genetics and biochemistry hold great promise for the future. As a result of these developments, classical nosology also in neurology changed, and new nosological entities arose not primarily based on pathological criteria.

Leigh syndrome constitutes an example of a neurological disorder to which newly developed techniques have brought new insights. Originally described as a progressive neurological degenerative disorder of unknown etiology, the diagnosis was based on pathologic criteria (subacute necrotizing encephalomyelopathy). It is nowadays incorporated in the, also biochemically characterized, mitochondrial encephalomyopathies. In the future it will probably be possible to classify these degenerative and progressive disorders according to their DNA pathology.

Despite all these technical developments the clinician's diagnostic efforts are still guided initially by patient history, and observation of clinical signs and symptoms. This clinical nosology must be integrated in new classification systems.

With expanding knowledge the fabric of medical science steadily becomes more complex and intricate. Although it has become impossible for the clinician to master the whole field of basic sciences that bring out the new developments, it is the task of the clinician to evaluate and integrate these new developments into the medical practice of every day. With this concept in mind, the present study of Leigh syndrome has been undertaken by a practising neurologist to bring together and integrate established knowledge and new insights and concepts to a state of the art.

CHAPTER I

INTRODUCTION, AIM AND OUTLINE OF THE STUDY

LEIGH SYNDROME: A MITOCHONDRIAL ENCEPHALO-MYOPATHY

The role of mitochondria in human pathology remained obscure during a long time. The first report of a patient with a myopathy and an abnormal mitochondrial function was published in 1959.[1] In 1962, Luft et al[2] reported on the same patient, a 35-year-old woman who suffered from an euthyroid hypermetabolism and a mild myopathy. In muscle biopsy they found morphologically abnormal mitochondria and a defective mitochondrial function due to uncoupling of respiration and phosphorylation in these mitochondria. Price[3] used the term mitochondrial myopathy to indicate a clinical condition characterized by muscle weakness and fatigue, associated with morphological or biochemical mitochondrial abnormalities.

The histopathological hallmark of the mitochondrial myopathies is the ragged-red fiber.[4] The ragged-red appearance consists of subsarcolemmal and intermyofibrillary purplish blotches on the modified Gomori trichrome stain,[5] caused by abnormal accumulation of mitochondria. Excessive accumulation of lipid droplets may overshadow the mitochondrial changes. In the past, much attention has been paid to the often peculiar morphological mitochondrial alterations, such as abnormalities of mitochondrial cristae or paracrystalline inclusions in the intramitochondrial space.[6] However, classification of mitochondrial myopathies based only on clinical and morphological criteria was unsatisfactory.

With the finding of biochemical errors of energy metabolism in several types of mitochondrial myopathies a classification based on biochemical abnormalities has appeared:[7–9]

1. defects of substrate transport, e.g. carnitine deficiency and carnitine palmitoyltransferase deficiency,
2. defects of substrate utilization, e.g. pyruvate dehydrogenase complex deficiency,
3. defects of the respiratory chain, e.g. NADH dehydrogenase deficiency or cytochrome *c* oxidase deficiency,
4. defects of the energy transducing and conserving system, e.g. hypermetabolic myopathy or myopathies with 'loose coupling'.

In the clinical spectrum of mitochondrial myopathies three subgroups have been identified:[10]

1. chronic progressive external ophthalmoplegia and limb weakness,
2. proximal weakness with fatigability,
3. predominantly or exclusively central nervous system disease.

The fact that in some patients with a mitochondrial myopathy also a central nervous system involvement was present, led Shapira et al[11] to propose

the concept of mitochondrial encephalomyopathies: a group of complex multisystem disorders with structurally and/or functionally abnormal mitochondria in brain and/or muscle. The group of mitochondrial encephalomyopathies encompasses the syndromes of Leigh,[12] of Alpers,[13] of dysmyelination,[9] of myoclonus epilepsy with ragged-red fibers (MERRF),[14] and of mitochondrial myopathy, encephalopathy, lactic acidosis and strokelike episodes (MELAS).[15]

Leigh syndrome or subacute necrotizing encephalomyelopathy is a progressive and often fatal neurological disorder, preferentially affecting young children.The clinical picture of Leigh syndrome shows a great variability. The diagnosis is still based on well-defined neuropathological criteria, but because a biopsy of the afflicted central nervous system areas is not feasible during life, the diagnosis can only be made by postmortem examination of the central nervous system. In the last decade Leigh syndrome was found to be associated with several defects of pyruvate metabolism or of the respiratory chain, and therefore it can be classified in the group of mitochondrial encephalomyopathies.

AIM AND OUTLINE OF THE STUDY

This study intends to provide a review of the literature data concerning Leigh syndrome and to present results of own investigations in patients with Leigh syndrome, with special emphasis on the clinical and biochemical aspects of the disorder.

The most important questions this study aims to answer are:

1. Can a better characterization of the clinical picture of Leigh syndrome be achieved?
2. Is it possible to distinguish clinically distinct subgroups within the spectrum of Leigh syndrome?
3. What is the contribution of technical (neurophysiological, neuroradiological and biochemical) investigations to diagnosis and to insight into the pathophysiology of Leigh syndrome?
4. Is it possible to formulate better criteria for a diagnosis of Leigh syndrome durante vitam?

The results of a literature study of 173 patients with proven Leigh syndrome are presented in Chapter II. This extensive survey provides data on clinical, genetic, biochemical and therapeutic aspects of the syndrome. In the other chapters (III-X) of our study the results of own investigations are presented. Within our patient group it was possible to distinguish a clinical subgroup with a distinct picture of parkinsonism in 3 patients. The value of

neurophysiological studies in Leigh syndrome was assessed in 12 patients (Chapter IV) and the value of an intravenous pyruvate loading test in 9 patients (Chapter V). Two patients with a proven and one patient with a suspected NADH dehydrogenase deficiency, as well as one patient with a partial cytochrome *c* oxidase deficiency (all in muscle biopsy material) are reported (Chapters VI-IX), and in one family a defect in oxidative metabolism was restricted to brain (Chapter X). Comparison of our results with literature data, contributions of our publications to the general concept and diagnosis of Leigh syndrome, and aspects of pathophysiology and antenatal diagnosis as well as future perspectives are discussed in Chapter XI.

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CHAPTER II

LEIGH SYNDROME
A Review of the Literature

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ABSTRACT

Results of a literature survey of 173 patients with Leigh syndrome are presented, with emphasis on signs and symptoms in relation to age at onset, contributions of technical investigations to the diagnosis, pathophysiology, genetic considerations and therapeutic aspects. Based on this study we are of the opinion that it is possible to come to a diagnosis of 'most probable Leigh syndrome' durante vitam on the combination of clinical signs and symptoms, autosomal recessive mode of inheritance, association with a defect of energy metabolism, and CT or MRI abnormalities.

INTRODUCTION

Since the first description by Dennis Leigh in 1951,[1] over 200 case reports of subacute necrotizing encephalomyelopathy (SNE) or Leigh syndrome have been published. The pathology of SNE is well-documented.[2,3] Clinical signs and symptoms, and course of the disease show considerable variability and do not allow a diagnosis of Leigh syndrome. Although in recent years several biochemical abnormalities of energy metabolism[4-7] that were associated with and possibly causally related to Leigh syndrome have been elucidated, the definite diagnosis still rests on pathologic findings.

We performed a literature survey of 173 cases of pathologically proven Leigh syndrome,[1-6,8-111] with emphasis on clinical presentation, results of neurophysiological, radiological, chemical and biochemical investigations, pathophysiology, genetics and therapy. First a synopsis of the pathology of Leigh syndrome is given, and then the results of our study will be presented.

PATHOLOGY

The lesions, that usually destroy gray and white matter without predilection, may be evident on macroscopic examination as focal gray-brown discolorations or cystic softenings in brainstem and medulla oblongata, or bilaterally symmetrical 'butterfly' lesions in periaqueductal region and tegmentum.[2] These often sharply punched-out lesions may be seen in various parts of the central nervous system (CNS). The incidence of lesions with regard to the main anatomical divisions of the central nervous system in the cases reviewed by Montpetit et al[3] is as follows: brainstem 98%, cerebrum 92%, spinal cord 74% and cerebellum 58%. The structures that show the highest incidence of lesions are brainstem tegmentum (92%), basal ganglia (67%), visual system (65%), substantia nigra (64%) and thalamus (51%). Apart from the tegmentum, brainstem lesions are often seen in midbrain tectum, ventral part of the medulla oblongata, and inferior olives. The spinal cord lesions are localized mainly in the anterior horns, dorsal columns and pyramidal tracts (Fig 1). In the cerebellum, dentate nucleus and corpus medullare show the highest incidence of lesions. Lesions rarely occur in cerebral or cerebellar cortex and mamillary bodies.

Histopathologically the changes consist of spongy necrosis with glial reactions and vascular proliferation. The lesions show a spongy loosening and vacuolation or even microcystic disintegration of the nervous parenchyma. Breakdown of myelin is almost constantly present in the lesions with microcystic spongiosis. A microglial reaction accompanies the disintegration of gray and white matter. A moderate to marked increase of

cytoplasmic and gemistocytic astroglia and a loss of oligodendroglia are present in most of the lesions. The vessels are dilated and there is an increase in the number of capillaries and precapillaries often with endothelial proliferation. Nerve cells and axons are remarkably preserved in the necrotic areas (Fig 2).

Ultrastructural studies of the CNS reveal axonal swelling and degeneration, loosening of myelin lamellae with formation of vacuoles, and hypertrophy and hyperplasia of capillaries with endothelial proliferation.[83,84,112] Structural mitochondrial abnormalities of the CNS have, to our knowledge, never been reported in Leigh syndrome.

In peripheral nerve signs of myelin loss and axonal degeneration have been described.[16,30,40,52,82]

In muscle 'ragged-red' fibers have been reported in 2 patients.[75,105] On electronmicroscopy of skeletal muscle of these patients accumulation of mitochondria and mitochondrial enlargement, proliferation of cristae and paracrystalline inclusions are seen. In another patient mitochondrial enlargement was reported in heart muscle.[113]

Rutledge et al[108] reported a series of 12 patients with Leigh syndrome of whom 7 had a hypertrophic cardiomyopathy, an infrequent abnormality in the other case reports.[11,12,18,40,75,84,113,114]

The CNS lesions in Leigh syndrome closely resemble those of Wernicke's disease in topographical distribution and histopathology. In Leigh syndrome, however, hemorrhage is very rare and the mamillary bodies are rarely involved, and the substantia nigra and basal ganglia are often affected, whereas the opposite holds true for Wernicke's disease. But on morphological grounds it is not possible to come to a definite distinction between the two entities.[76]

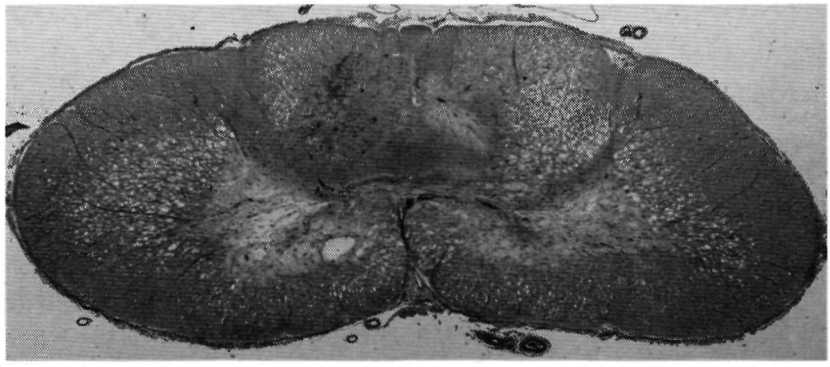

Fig 1. Spinal cord (hematoxylin-eosin, x8.3). Extensive microcystic degeneration.

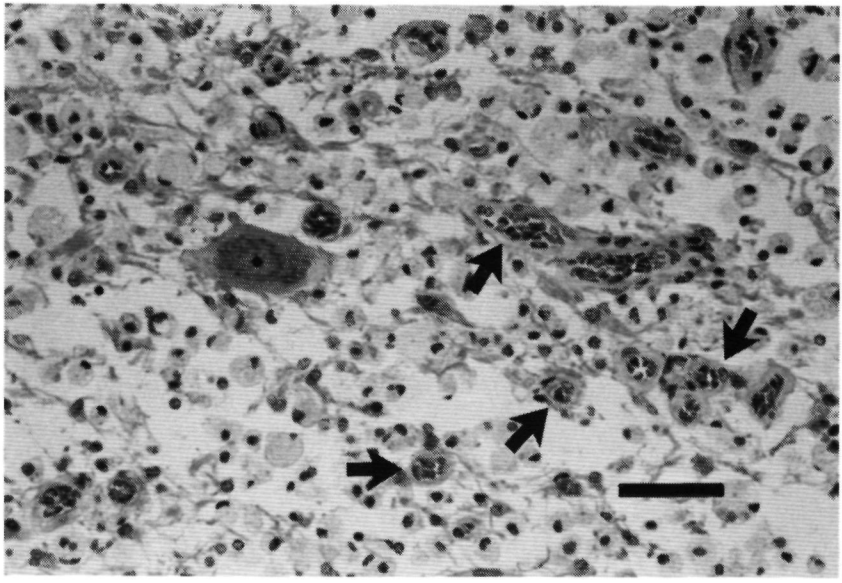

Fig 2. Detail anterior horn (hematoxylin-eosin, bar = 50 μm). Spongy dystrophy, vascular proliferation (arrows) and relative sparing of motor neuron cell.

CRITERIA OF SELECTION OF LITERATURE PATIENTS

We selected a total of 173 patients. In 165 patients the diagnosis was proven on pathologic examination, and in 8 patients the diagnosis was confirmed in a sib. Age at onset, clinical signs and symptoms, and course of the disease had to be adequately described.

We divided the 173 patients in 4 groups according to age at onset: neonatal (n=20; 0-4 weeks), early infantile (n=81; 4 weeks-1 year), infantile (n=48; 1-4 years) and juvenile (n=24; 4-16 years). Adolescent (n=1)[114] and adult (n=13)[95,112,115-121] patients were not included in the study because in some of them there were serious doubts considering the correctness of the diagnosis.[118] Pathology, clinical signs and symptoms, and course of the disease in the adolescent and adult patients, however, seem essentially the same as in the juvenile patients.

SEX RATIO

Numbers of patients and sex ratios in each group are shown in Table 1. There is a preponderance of males over females, that becomes more

pronounced with increasing age at onset. In the adult onset patients, however, the sex ratio is 1.0. As the mode of inheritance is presumably autosomal recessive (see 'genetic considerations'), equal numbers of male and female patients would be expected. The sex ratios of the first three age groups are, more or less, compatible with this hypothesis ($p = 0.49$, 0.18 and 0.18 respectively, sign test), but that does not hold true for the juvenile group ($p = 0.01$, sign test).

Table 1. Age distribution and sex ratio of 173 Leigh patients from the literature

		n	(%)	M : F
Neonatal	0 - 4 w	20	(12)	2 : 3
Early infantile	4 w - 1 y	81	(46)	3 : 2
Infantile	1 - 4 y	48	(28)	3 : 2
Juvenile	4 - 16 y	24	(14)	4 : 1
Total	0 - 16 y	173		3 : 2

M = male; F = female.

AGE AT ONSET AND SURVIVAL

Leigh syndrome presents preferentially in very young children (median age at onset 0.8 years) (Table 1). The age at death, documented in all deceased patients (n=165) has a median of 2.1 years. In Table 2, median time of survival after onset of symptoms is presented. The duration of the disease actually ranges between a few hours (in the neonatal and early infantile groups) and one or more decades (Table 2).

Table 2. Median and maximum time of survival (duration of illness) after onset of disease

	Median survival*			Maximum survival*
	M+F	M	F	M+F
Neonatal	0.9	1.0	0.6	9.0
Early infantile	0.9	0.9	0.8	19.5
Infantile	0.9	0.9	0.9	11.5
Juvenile	5.5	4.8	8.0	33.0

*years; M = male; F = female.

SYMPTOMATOLOGY AT ONSET

Signs and symptoms at onset of 169 patients are summarized in Table 3. Feeding problems encompass dysphagia, inadequate sucking, regurgitation, anorexia and periodic vomiting. It can be a presenting complaint, especially in the younger children, or develop later in the course of the illness and is even quite often a terminal phenomenon. Eye signs and

symptoms consist of blindness, optic atrophy, diminished pupillary reaction to light, mild ophthalomoplegia, strabismus, nystagmus and jerky eye movements. Irregular respiration and periods of hyperventilation, apnea and frequent sighing are the main respiratory problems. Ataxia, dysdiadochokinesis, hypermetria and explosive speech indicate cerebellar involvement. Extrapyramidal signs are hypokinesia, rigidity, dystonia, athetosis, choreoathetosis and ballism.

According to Pincus[122] the onset of the disease is insidious in 71%, subacute in 14% and acute in 15%. Seizures, sometimes status epilepticus, and coma are often seen in cases with acute presentations.

Table 3. Signs and symptoms at onset of Leigh syndrome (%)

Feeding problems	28
Motor retardation	27
Hypotonia	22
Eye signs and symptoms	18
Mental retardation	12
Seizures	8
Respiratory problems	7
Cerebellar signs	7
Pyramidal signs	5
Extrapyramidal signs	3
Coma	2

THE COURSE OF THE DISEASE

The course of the disease[122] is chronic and unremitting in 55%, chronic and remitting in 28%, subacute and unremitting in 15%, and acute and unremitting in 2%.

In Table 4, symptomatology in the course of the disease of all patients is summarized. Height is below the 10th percentile in 17% of the children and skull circumference in 5%. The most frequent signs and symptoms are presented for the different age groups. It can be seen from Table 4 that eye signs and symptoms show the same incidence in all age groups. Pyramidal, cerebellar, extrapyramidal and cardiac signs show an increasing incidence with increasing age. Respiratory problems, motor retardation and feeding problems show a gradual decrease in incidence with increasing age at onset. Deafness, that is often considered one of the clinical hallmarks of Leigh syndrome, is encountered in only 7% of the patients. Disturbances of cardiac rhythm constitute the main part of cardiac signs and long lasting periods of tachycardia and bradycardia are reported. The hypertrophic cardiomyopathy[108] is often asymptomatic or only a systolic murmur is heard.

As the cause (or one of the causes) of death a neurogenic disturbance

Table 4. Signs and symptoms in the course of the disease in relation to age at onset

	Neonatal (n=20)	Early infantile (n=81)	Infantile (n=48)	Juvenile (n=24)	All age groups (n=173)
Eye signs and symptoms	80%	77%	81%	79%	78%
ophthalmoplegia	25%	31%	46%	42%	36%
nystagmus	30%	32%	31%	33%	32%
optical atrophy	25%	26%	25%	50%	29%
loss of vision	25%	21%	15%	42%	23%
diminished pupillary reaction	25%	21%	21%	4%	19%
abnormalities of retina/macula	5%	5%	4%	17%	6%
Respiratory problems	80%	68%	77%	54%	69%
irregular respiration	30%	56%	54%	33%	49%
apnea periods/terminal	50%	24%	35%	21%	30%
hyperventilation	15%	11%	13%	8%	12%
Hypotonia	60%	68%	81%	58%	69%
Pyramidal signs	65%	60%	48%	88%	61%
extensor response	30%	28%	35%	38%	32%
hyperreflexia	40%	35%	25%	29%	32%
hypertonia	40%	26%	15%	42%	27%
paresis	25%	6%	23%	46%	18%
Motor retardation/deterioration		68%	54%	29%	58%
Feeding problems	60%	59%	52%	42%	55%
anorexia	55%	43%	29%	17%	37%
vomiting	35%	35%	35%	33%	35%
weight loss	50%	35%	25%	8%	30%
Exercise intolerance/fatigue	60%	40%	54%	50%	47%
Infections (decompensation/deterioration)	25%	44%	46%	42%	39%

Table 4. (continued)

	Neonatal (n=20)	Early infantile (n=81)	Infantile (n=48)	Juvenile (n=24)	All age groups (n=173)
Cerebellar signs		22%	65%	54%	39%
ataxia	20%	20%	56%	46%	34%
dysarthria		4%	15%	33%	10%
Mental retardation		42%	31%	50%	37%
Seizures	30%	45%	25%	33%	36%
generalized	15%	36%	17%	25%	27%
partial (focal)	20%	20%	10%	17%	17%
Extrapyramidal signs		21%	19%	46%	24%
rigidity/akinesia		6%	10%	25%	9%
chorea		9%	6%	13%	8%
athetosis/dystonia		6%	2%	13%	6%
tremor		1%		4%	2%
Cardiac signs and symptoms	10%	15%	21%	25%	18%
Deafness/hearing loss	5%	10%	2%	8%	7%
Sensibility disorder		3%	6%	12%	5%

of respiration is mentioned in 44%, sudden coma e.c.i. in 26%, pneumonia and cardiac disorder both in 12%, and hyperpyrexia in 3%. The cause of death is stated unknown in 36%.

TECHNICAL INVESTIGATIONS

NEUROPHYSIOLOGICAL STUDIES

In 50% of the patients described in literature, results of electroencephalography (EEG) and in 18% results of electromyography (EMG) and nerve conduction velocity studies are mentioned. Evoked potentials (VEP/BAEP) are recorded in only 5 patients. The three patients mentioned by Hecox et al[123] of whom two had BAEP abnormalities, are not included in our series of 173 patients because description of the clinical data of the patients is lacking.

The neurophysiological findings in the literature patients are presented in Table 5. As can be seen from this table there are no constant or specific EEG abnormalities. Diffuse slowing and epileptic manifestations of various nature are seen most often. Nerve conduction velocities, measured in 24 patients, are reduced in 11 (46%). In only a few patients (3/22) EMG shows a myopathic picture. Evoked potentials are normal in 2 and abnormal in 3 patients.

RADIOLOGICAL INVESTIGATIONS

In a number of Leigh patients it is possible to demonstrate the cerebral lesions on CT scan and magnetic resonance imaging (MRI). MRI is much more sensitive than CT scan, in that it can visualize multifocal abnormalities in some cases, where CT scan fails to detect any abnormal findings.[124–126].

The most constant finding in Leigh syndrome is that of bilateral radiolucent or hypodense regions in the basal ganglia, most often localized in the putamina.[88,97,103,126–130]. Bilateral symmetrical low density areas in the thalamus, throughout the midbrain and adjacent to the fourth ventricle and scattered throughout the cerebral cortex have also been reported.[103,125] These findings are dynamic and may vary with time.[131]

Bilateral low density areas in the lentiform nuclei are also encountered in other pathologic states, such as Wilson's disease,[132] postanoxic states,[133] carbon monoxide[134] and methylalcohol poisoning,[135] and (familial) striatonigral atrophy.[88]

CT/MRI abnormalities in the basal ganglia region do not necessarily clinically present with extrapyramidal signs. This fits in with the finding

Table 5. Electrophysiological studies in 173 Leigh syndrome patients in the literature

EEG (n=87):	Normal			31
	Diffuse slowing			29
	Focal slowing			6
	Increase of amplitude			4
	Dysrhythmia			2
	Epileptic manifestations			17
	- generalized spikes		3	
	- paroxysms		4	
	- focal spikes		4	
	- spike and wave complexes		4	
	- hypsarhythmia		2	
EMG (n=31):	Electromyography:	Normal		17
		Denervation		2
		Myopathy		3
	Nerve conduction velocities:	Normal		13
		Reduced:	motor+sensory	11
			motor	3
			sensory	1
VEP (n=4):	Normal			2
	Not elicitable over right occipital cortex			1
	Not elicitable			1
BAEP (n=1).	Right ear: normal, left ear: not elicitable			(1)

of pathologic lesions in the basal ganglia in 65% of the cases in the series of Montpetit et al[3] while only 12% of the patients manifested extrapyramidal signs.

CHEMICAL AND BIOCHEMICAL STUDIES

In 1965, Worsley et al[33] described lactic acidosis in familial Leigh syndrome. In the series of 173 patients, serum pyruvate levels are measured in only 21 patients and show an increase in 20 patients. Serum lactate levels are increased in 27 out of 33 patients, and serum alanine levels in 10 out of 12 patients. Cerebrospinal fluid (CSF) pyruvate, lactate and alanine levels show an increase in 5 out of 5, 5 out of 6, and 6 out of 6 patients respectively. In CSF there is an increase in protein level in about half of the patients. In Table 6 the most important chemical findings in serum, CSF and urine are summarized.

Since the first report by Hommes et al[4] of a pyruvate carboxylase (PC) deficiency in liver associated with Leigh syndrome, there have been several reports on deficiencies of pyruvate metabolism and respiratory chain defects in liver, muscle, kidney, brain and fibroblasts in Leigh patients. Several other authors[110,136,137] have linked PC deficiency with pathologically proven

Table 6. Results of biochemical studies in Leigh syndrome

	Serum	CSF	Urine
Increased pyruvate level	20 (21)	5 (5)	3 (5)
Increased lactate level	27 (33)	5 (6)	1 (2)
Increased alanine level	10 (12)	3 (4)	6 (6)
Increased protein level		41 (85)	

In parentheses the total number of patients in whom the results of the particular chemical study were mentioned.

SNE, but methods and interpretation of findings remain controversial.[138,139] Deficiencies of the pyruvate dehydrogenase complex (PDHc),[5,106,107,109,140,141] a defective activation of PDHc[94,142] and defects of the respiratory chain, i.e. cytochrome *c* oxidase[6,143,144] and NADH-CoQ oxidoreductase,[7] have been found in association with Leigh syndrome (Fig 3). Deficiencies of pyruvate metabolism or respiratory chain defects restricted to the CNS have never been reported in Leigh syndrome. All the deficiencies reported in Leigh syndrome (PC, PDHc, cytochrome *c* oxidase and NADH-CoQ oxidoreductase) have in common that even a severe deficiency can occur without evidence of SNE.[145–148]

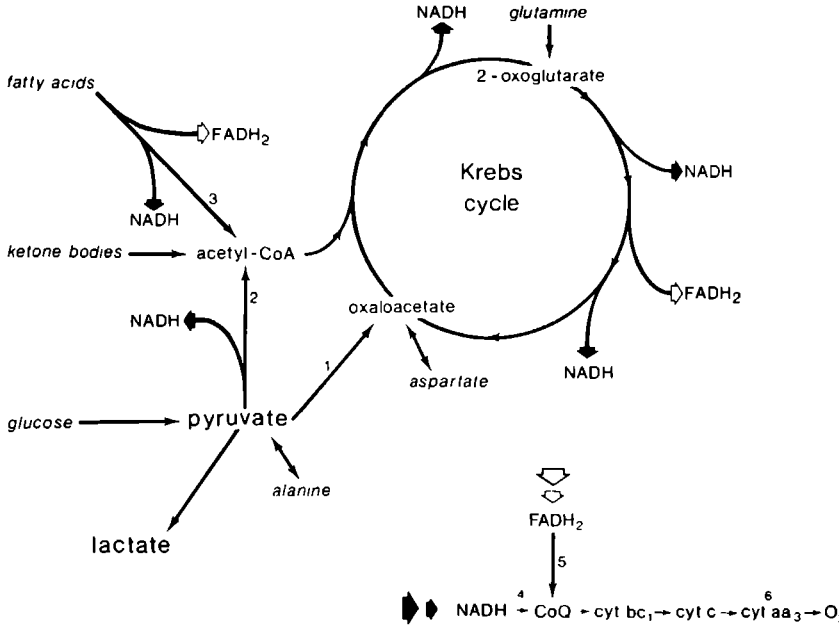

Fig 3. The formation of pyruvate from nutrients, and the role of the Krebs cycle and respiratory chain in the catabolism of pyruvate (see text for further explanation).
1 = pyruvate carboxylase, 2 = pyruvate dehydrogenase complex, 3 = β-oxidation of fatty acids, 4 = NADH dehydrogenase, 5 = $FADH_2$ oxidizing proteins, 6 = cytochrome *c* oxidase.

PC, PDHc and the respiratory chain are located in the mitochondria and their deficiencies give rise to a disturbance of mitochondrial metabolism. Mitochondrial dysfunction is the common denominator in the so-called mitochondrial myopathies,[149,150] a clinically and biochemically heterogeneous group of disorders characterized by structurally or numerically abnormal mitochondria or by abnormal functioning of mitochondria. In the clinical spectrum of the mitochondrial myopathies three syndromes can be distinguished:[151] 1) chronic progressive external ophthalmoplegia (CPEO) and limb weakness, 2) proximal weakness with fatigability, and 3) predominantly or exclusively CNS disease. As it became generally recognized that CNS disease is a prominent feature in a subgroup of the mitochondrial myopathies, Shapira et al[152] expanded the concept of mitochondrial myopathy to mitochondrial encephalomyopathy. The mitochondrial encephalomyopathies show CNS and muscle involvement, associated with abnormalities in mitochondrial metabolism and/or morphology. Leigh syndrome is one of these mitochondrial encephalomyopathies. Other representatives of this group are the syndromes of Alpers,[153] dysmyelination,[150] myoclonus epilepsy associated with ragged-red fibers (MERRF),[154] and the so-called MELAS syndrome,[155] which stands for mitochondrial myopathy, encephalopathy, lactic acidosis and strokelike episodes.

The close resemblance of the lesions in SNE and Wernicke's disease prompted research of thiamine metabolism in Leigh syndrome. In 1969, Pincus et al[55] discovered *in vitro* inhibition of the mitochondrial enzyme thiamine pyrophosphate-adenosine triphosphate phosphotransferase by urine and serum of a patient with SNE. Thiamine triphosphate (TTP) was absent in the brain of their patient and they presumed this was caused by a so-called 'inhibiting factor'. Later the same authors reported low values of TTP in SNE brains, roughly correlating with the sites of the brain lesions.[156] The urine inhibitor test, however, failed to prove a specific biochemical marker for antemortem diagnosis of Leigh syndrome because it has a 6% false-positive rate, and no inhibiting factor could be demonstrated in 6 out of 29 patients.[157]

GENETIC CONSIDERATIONS

Leigh syndrome is generally considered to be a familial degenerative disorder with an autosomal recessive mode of inheritance.[158,159] Arguments in favour of an autosomal recessive mode of inheritance in our patient group are a parental consanguinity in 8 families and the familial occurrence of Leigh syndrome in both sexes in 56 families, in which only one generation is afflicted. In these 56 families there are 56 probands, 53 sibs who are

also affected, and 108 healthy sibs. When the index cases are omitted, 33% (53/161) of the sibs in these families are affected, which is significantly more ($p = 0.01$) than would be expected in autosomal recessive inheritance. The reason for this increased number of affected sibs is not clear to us. There is a bias of ascertainment because those families with 2 or more affected sibs are more likely to be published. In 12 families with healthy and affected sibs, the sex of the affected children is recorded and there is a boy-girl ratio of 1.0. An X-linked mode of transmission was postulated by Benke et al[104] in two halfbrothers with Leigh syndrome who had different fathers. The existence of X-linkage in some cases of Leigh syndrome might explain the preponderance of males in our series (see 'sex ratio'). In the same sibship, cases with a different age at onset occur and thus there are no genetic grounds for a division of Leigh syndrome in four age groups according to age at onset.

An interesting new perspective in the transmission of certain respiratory chain defects is formed by the maternal inheritance of mitochondrial defects.[160] Some of the components of the respiratory chain, e.g. subunits of cytochrome *c* oxidase are coded for by mitochondrial DNA.[161] In the formation of the zygote almost all the mitochondria are contributed by the ovum.[162] So, mitochondrial properties are inherited from the mother. This maternal or mitochondrial type of inheritance can be distinguished from the Mendelian type of inheritance by involvement of subsequent generations with a higher number of affected individuals in each generation than expected in autosomal dominant diseases, and involvement of both sexes in contrast to X-linked recessive transmission.[163] Maternal inheritance has not yet been conclusively documented in Leigh syndrome, but it is probable that it is present in some cases.[164] Maternal inheritance has been reported in MERRF syndrome,[154,165] one of the mitochondrial encephalomyopathies.

THERAPEUTIC CONSIDERATIONS

No causal treatment of the condition is available so far, and no controlled trials have been performed with substances or drugs that were reported to be benificial in patients with disturbances of pyruvate metabolism or the respiratory chain, clinically presenting as mitochondrial (encephalo)-myopathies. Apart from general measures as a low carbohydrate diet to prevent exacerbations from carbohydrate sensitivity in PC and PDHc deficiencies,[166] the therapeutic efforts in patients with disturbances of pyruvate metabolism or respiratory chain are aimed at circumventing the deficient enzyme, or at optimizing enzyme function by pharmacological doses (of precursors) of cofactors.

In ketotic states (starvation, high fat ketogenic diet), brain tissue can utilize ketone bodies produced by the liver in direct proportion to their concentrations in the arterial blood.[167] Ketogenic diet provides clinical and biochemical improvement in some patients with PC and PDHc deficiencies.[166,168,169]

In PC deficiency, administration of additional glutamine and aspartate, both precursors of oxaloacetate, can be helpful.[136,147,170] Administration of the PC cofactor biotin seems to be ineffective.[66,171]

In PDHc deficiency, administration of thiamine (a cofactor of the first enzyme of the PDH complex) and α-lipoic acid (a cofactor of the second enzyme of the PDH complex) can be considered. These compounds, alone or in combination, have been administered with variable results.[4,36,168,169,172,173]

Recently, favourable results have been reported from therapeutic efforts in respiratory chain deficiencies.[174,175] Riboflavin, a precursor of the flavin moiety of flavoproteins, appears to be of benefit in patients suffering from a reduced activity of one of the many flavoproteins, for example in patients with a NADH-CoQ oxidoreductase deficiency[176] and in patients with a multiple acyl-CoA dehydrogenase deficiency (glutaric aciduria type II).[177]

FINAL REMARKS

We performed a literature study on 173 pathologically proven Leigh patients. The sex ratio of 4 : 1 (Table 1) in the juvenile onset group deviates significantly from the expected 1 : 1. We have no explanation for this phenomenon, nor for the preponderance of males in the whole group of 173 patients. X-linked inheritance, that might explain this male excess, has only been documented once in Leigh syndrome, and our findings confirm the autosomal recessive mode of inheritance of Leigh syndrome.

Signs and symptoms show a great variability, which is not surprising considering the variable topography of the pathologic lesions. However, there is a cluster of signs and symptoms (Tables 4 and 5) that, although not pathognomic, is encountered often in Leigh syndrome. The division of the patients in this study in 4 groups according to age at onset, does not intend to suggest that there exist distinct neonatal, early infantile, infantile and juvenile forms of Leigh syndrome. Clinical signs and symptoms, the results of biochemical studies and the lack of true breeding do not support the concept of distinct age-related subgroups of Leigh syndrome. The group with a juvenile age at onset, however, can be separated from the rest in that the disease in these children runs a much more protracted course and median survival is much longer than in the other groups (Table 2).

Neurophysiological studies do not contribute to the diagnosis. Cerebral CT scan and MRI can show bilateral hypodense areas in the basal ganglia region.

Leigh syndrome has been firmly associated with a number of deficiencies in the mitochondrial energy metabolism. Morphological or functional mitochondrial abnormalities are characteristic of the group of mitochondrial myopathies and one of its subgroups, with predominantly or mainly CNS disease, the mitochondrial encephalomyopathies. Leigh syndrome belongs to this latter group.

A disturbance of pyruvate metabolism or a deficiency of the respiratory chain often lead to an elevation of the levels of lactate and pyruvate in serum, CSF and urine. Determination of the levels of lactate and pyruvate in body fluids is therefore of utmost importance when a patient is suspected of Leigh syndrome (Table 6).

In the future much more insight into the pathogenesis of the mitochondrial (encephalo)myopathies will be gained when the biochemical defects can be identified at a molecular level. Another promising development is that of *in vivo* phosphorus magnetic resonance spectroscopy (^{31}P-NMR), that has proven useful in the noninvasive diagnosis of mitochondrial myopathies, in monitoring therapeutic improvements in mitochondrial myopathies,[178] and in defining the pathophysiological basis of these disorders.[179]

Despite all recent developments, the definite diagnosis of Leigh syndrome still rests on pathologic findings. But, in our opinion, it is possible to come to a clinical diagnosis of most probable Leigh syndrome on the basis of the following criteria: 1) signs and symptoms, 2) autosomal recessive mode of inheritance, 3) disturbance in energy metabolism, 4) bilateral hypodense areas in the basal ganglia region on CT or MRI.

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STUDIES IN PATIENTS WITH LEIGH SYNDROME

CHAPTER III

HYPOKINESIA AND RIGIDITY AS THE CLINICAL MANIFESTATIONS OF A MITOCHONDRIAL ENCEPHALOMYOPATHY

Report of 3 Cases

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ABSTRACT

Three patients are reported with a neurological disorder in whom hypokinesia and rigidity are the most prominent clinical manifestations. On CT-scan and MRI, two patients show bilateral lesions in the striatum, mainly in the putamen, and the third patient shows bilateral lesions in the posterior limb of the internal capsule. Chemical investigations suggest a disturbance of pyruvate metabolism in all three patients. In skeletal muscle of two patients a disturbance of pyruvate oxidation was established. In one patient it was caused by a NADH dehydrogenase defect. The signs and symptoms, the bilateral lesions in the striatum in two patients, and the association with a disturbance of pyruvate metabolism justify classification of these patients as mitochondrial encephalomyopathies, resembling Leigh syndrome. Therefore in infantile and juvenile patients presenting with parkinsonism, a mitochondrial encephalomyopathy must be considered and the mitochondrial energy metabolism should be investigated.

INTRODUCTION

The mitochondrial encephalomyelopathies constitute a group of neuromuscular disorders associated with defects in mitochondrial metabolism or structure (Shapira et al, 1977; Gabreëls et al, 1984). Representatives of this group are the syndromes of Leigh (Hommes et al, 1968; Farmer et al, 1973; Willems et al, 1977; Van Erven et al, 1985) and Alpers (Gabreëls et al, 1984), dysmyelination (Sengers et al, 1984), Kearns-Sayre syndrome (Karpati et al, 1973), myoclonus epilepsy with 'ragged-red fibres' (Fukuhara et al, 1980), and the MELAS syndrome, an acronym which stands for mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (Pavlakis et al, 1984). These syndromes vary considerably in clinical signs and symptoms, but they all show cerebral spongiosis, although to a varying extent and with different localisation (Walter, 1983).

The diagnosis of Leigh syndrome or subacute necrotizing encephalomyelopathy rests on characteristic pathologic findings in brainstem, spinal cord, and basal ganglia, especially the striatum (Jellinger and Seitelberger, 1970). Ante mortem diagnosis of probable Leigh syndrome can be made when clinical signs and symptoms indicate involvement of the above mentioned parts of the central nervous system (CNS) (Pincus, 1972) and when a disturbance of pyruvate metabolism is present (Van Erven et al, 1987). The presence of bilateral hypodensities in the basal ganglia in CT scanning or abnormal signal intensities in magnetic resonance imaging (Hall and Gardner-Medwin, 1978) can further support the clinical diagnosis.

In this report we present three patients with a mitochondrial encephalomyopathy and a clinical diagnosis of Leigh syndrome, in whom hypokinesia and rigidity constitute the initial and most prominent clinical manifestations.

CASE REPORTS

In none of the cases there is parental consanguinity. Family histories are unremarkable and do not contain evidence of neuromuscular or central neurological disorders.

Case 1

The pre-, peri- and postnatal periods of this boy are normal. Apart from difficulties in feeding, his early development is uneventful. He reaches motor milestones with some delay. At the age of 2 years he can walk alone, but is clumsy. At that age, exercise intolerance and slow recovery after infections (otitis, bronchitis) become apparent. Because of slowly progressive deterioration of motor abilities over the years he is presented at our department at age 7 years.

On examination marked hypokinesia with a stooped posture and semiflexion of the

extremities are striking Full scale IQ is normal Height is 123 cm (P50), weight is 20 kg (P10) and head circumference is 50 5 cm (P20) General examination is normal except for periods of paroxysmal tachypnoea and tachycardia There is a strabismus divergens of the right eye, a first-grade horizontal nystagmus on looking to the right, and a rotatory nystagmus on looking upwards. Fundoscopy is normal Speech is soft, slow, monotonous and somewhat slurred He shows a generalized rigidity of muscle tone with a mask-like face and a marked hypokinesia with start hesitation, but tremor is not apparent Dystonic movements of the trunk are occasionally seen. A cerebellar type of ataxia and dysmetria of the extremities are apparent. There is no sensory loss Tendon reflexes are hyperactive. Plantar reflexes show an extensor response. He is wheelchair-bound but can walk a few small steps

Case 2

This male patient is born after an uncomplicated pregnancy and delivery. Early psychomotor development is normal Motor milestones are reached in due time, but motor performance is always slow and clumsy At the age of 8 years, a deterioration of motor function appears He often falls and speech becomes soft and slurred. On intercurrent infections a marked deterioration of speech and motor function appears, followed by a slow recovery.

On examination at the age of 9 years we see a small, pale boy with thin, atrophic muscles, a mask-like face and a stooped posture with flexion of arms and legs, who functions on a normal mental level Height is 124 cm (P5), weight is 21 kg (P2 5) and head circumference is 53 cm (P50). General examination is normal except for frequent sighing and periods of tachy- and bradycardia. Visual acuity is sufficient, but fundoscopy reveals a bilateral optic atrophy. Speech is soft, slow, monotonous and slurred He walks with shuffling, small steps and has start hesitation There is a generalized rigidity of muscle tone with a cogwheel phenomenon, more marked on the left side Tremor is not apparent. The limbs are ataxic There are no sensory disturbances. Tendon reflexes are hyperactive, more markedly on the left, and plantar reflexes show an extensor response.

At the age of 14 years, only a slight progression of hypokinesia and rigidity is noted.

Case 3

This male patient's medical history is unremarkable until age 14 years when changes in his conduct with social withdrawal and aggressive behaviour, associated with a loss of motor abilities, exercise intolerance and deterioration on intercurrent infections, become manifest.

On examination at the age of 17 years, we see a small, thin boy with a maskhke face and a parkinson-like stooped posture with flexion of arms and legs, who functions on a normal mental level Height is 163 cm (P2.5), weight is 41 kg (P2 5) and head circumference is 52.5 cm (P10) Irregular breathing with frequent sighing and bouts of sinus tachycardia of 140 beats/min and sinus bradycardia of 50 beats/min are noted Fundoscopy is normal. There is a strabismus convergens of the right eye. Eye movements are jerky with loss of smooth pursuit. There is a marked hypokinesia, and a generalized rigidity with a cogwheel phenomenon. Tremor is not apparent, but the hands show athetotic movements. When walking, one remarks start hesitation, small steps and a mild broad-based gait. Speech is soft, monotonous and slurred. There is generalized muscular weakness. Ataxia and intention tremor indicate cerebellar involvement. There are no sensory disturbances. Tendon reflexes are hyperactive with bilateral extensor plantar responses.

TECHNICAL INVESTIGATIONS

Neurophysiological studies (electroencephalogram, electromyogram, nerve conduction velocity studies, visual evoked potentials, brainstem auditory evoked potentials and median nerve somatosensory evoked potentials) are normal in all patients.

Electrocardiography and echocardiography are unremarkable in our patients.

NEURORADIOLOGICAL INVESTIGATIONS

Neuroradiological investigation consists of cerebral computed tomography (CT) and magnetic resonance imaging (MRI) in all patients. A four-vessel angiography is performed in two patients, and is normal in both.

CT scans in patients 1 and 2 show bilateral symmetrical hypodense areas in the putamen. In patient 2 there is also diffuse central and cortical atrophy of the cerebral hemispheres and cerebellum. In patient 3 there are no abnormalities (Figs 1-3).

The MRI scans are performed on a super-conducting 0.5 Tesla Philips scanner, and are directed to the striatum because of the hypokinesia and rigidity of our patients. Axial sections are performed through the striatum using both T_1- and T_2-weighted pulse-sequences. In one section a mixed-mode sequence is used for determination of T_1 and T_2 relaxation times (IR: TE 50 ms, TI 500 ms, TR 1500 ms; SE: TE 50 ms, TR 1000 ms; four echos) (Mills et al, 1984; Orthendahl et al, 1984; Brown et al, 1985; Kjos et al, 1985).

MRI investigation of the striatum of patient 1 demonstrates hypo-intensive grey matter abnormalities bilaterally in the putamen and in the head of the right caudate nucleus on the calculated T_1 image. These abnormalities are hyper-intensive on the calculated T_2 image (Fig 1). In patient 2 identical lesions are found bilaterally in the putamen (Fig 2). In patient 3 the calculated T_2 image shows hyperintensive areas bilaterally in the posterior limb of the internal capsule (Fig 3). In Table 1 calculated T_1 and T_2 values of the putamen are compared with similar values of the thalamus. In children above age 2 years T_1 and T_2 values of cerebral tissue in a magnet of 0.5 Tesla are in the same range as in adults: 600 $\pm$ 100 ms and 100 $\pm$ 20 ms respectively (mean $\pm$ SD) (Bottomley et al, 1984; Bydder and Young, 1985; Komiyama et al, 1987).

LABORATORY INVESTIGATIONS

STANDARD INVESTIGATIONS

Results from blood and urinary studies, including haematologic evaluation, renal, hepatic, thyroid and adrenal functions, serum protein, serum protein electrophoresis and immunoelectrophoresis are normal. Other normal values include serum pH, pCO_2 and bicarbonate, serum lipids, short-chain

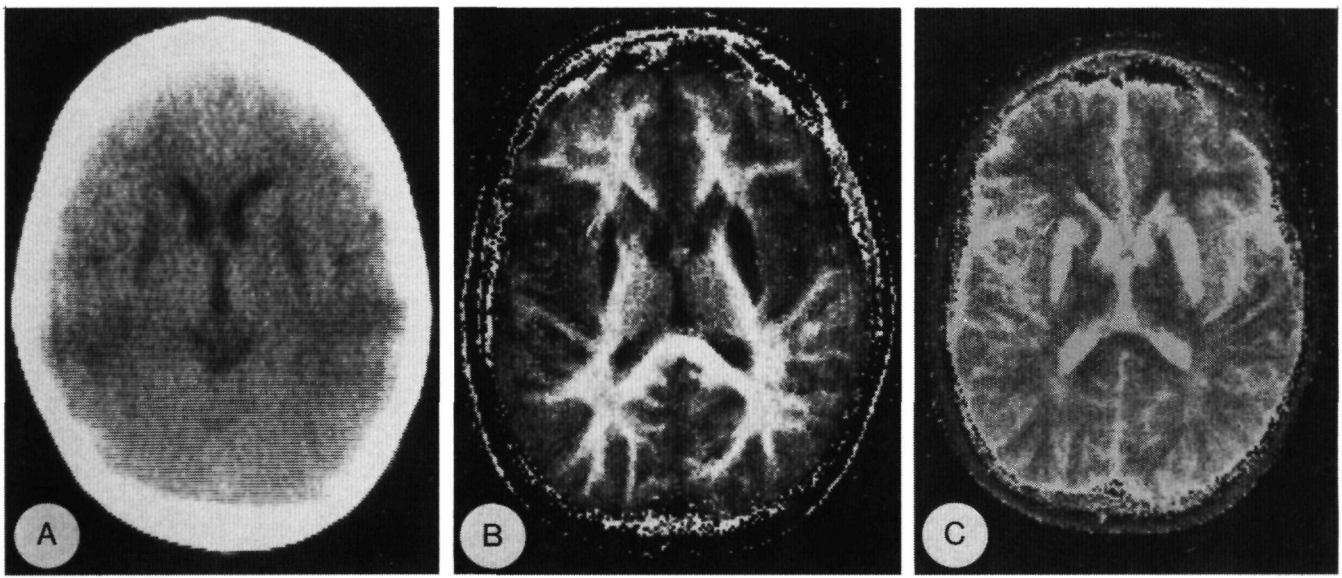

Fig 1. *(Case 1).* A, CT image. Hypodense areas in the putaminal regions. B, calculated T_1 image. Hypointensive areas in the putaminal regions and head of the right caudate nucleus. C, calculated T_2 image. Hyperintensive areas in the putaminal regions and head of the right caudate nucleus.

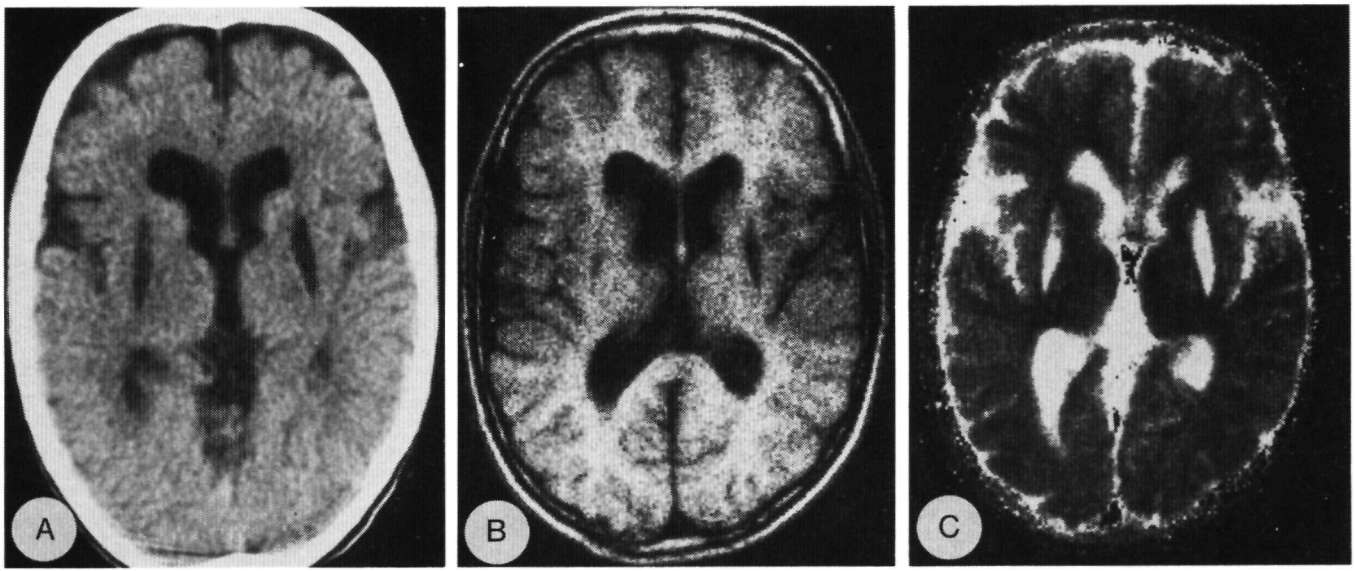

Fig 2. *(Case 2).* A, CT image. Hypodense areas in the putaminal regions. B, T_1-weighted image (SE: TE 30 ms, TR 400 ms). Hypointensive areas in the putaminal regions. C, calculated T_2 image. Hyperintensive areas in the putaminal regions.

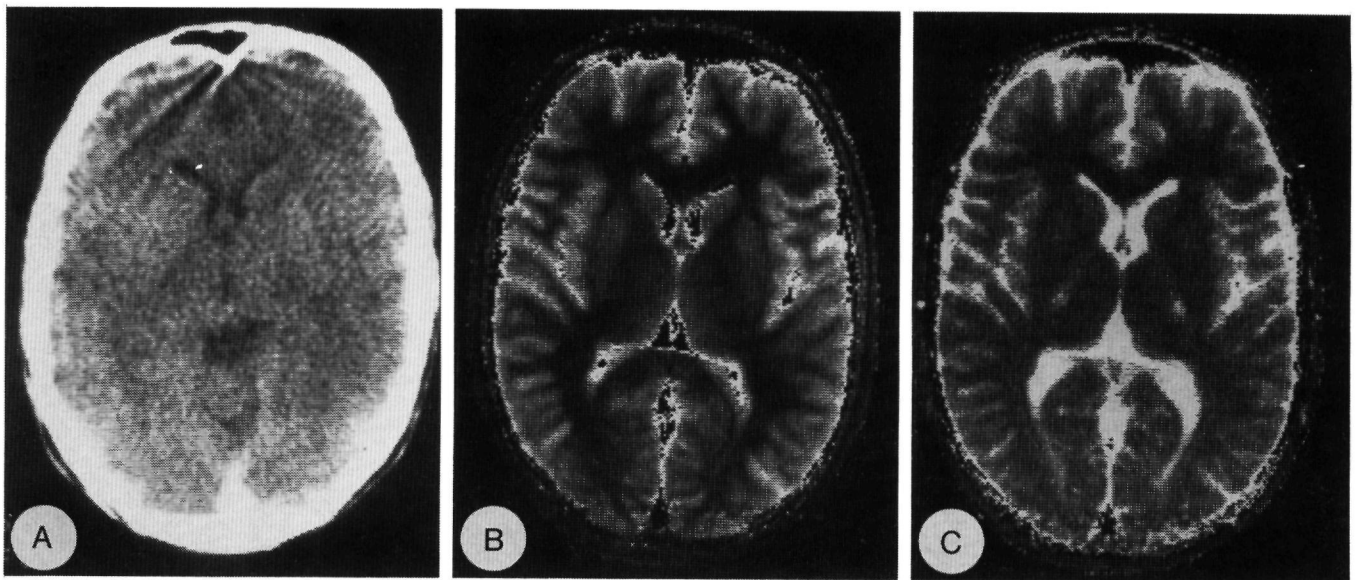

Fig 3. *(Case3).* A, CT image. Normal densities in the regions of the basal nuclei. B, calculated T_1 image. Normal intensities in the regions of the basal nuclei. C, calculated T_2 image. Hypointensive areas bilaterally in the posterior limb of the internal capsule.

Table 1. Calculated T_1 and T_2 relaxation times (in ms at 0 5 Tesla) in putamen and thalamus

			CT	MRI	T_1*	T_2*
Patient 1	thalamus	left	N	N	1087±100	86±6
		right	N	N	1035±136	86±6
	putamen	left	P	P	518±65	231±15
		right	P	P	590±196	215±27
Patient 2	thalamus	left	N	N	1428±451	90±9
		right	N	N	1382±358	95±8
	putamen	left	P	P	1479±232	370±38
		right	P	P	1430±135	336±115
Patient 3	thalamus	left	N	N	568±46	87±7
		right	N	N	527±50	89±5
	putamen	left	N	N	640±40	93±5
		right	N	N	690±41	91±7
Control subjects			N	N	600±100	100±20

N = normal imaging, P = pathological imaging, * values ± 1 SD

fatty acids and carnitine (total and free), growth hormone, thiamine, pyridoxine, cobalamine, creatine kinase, copper, zinc, manganese and caeruloplasmin, lysosomal enzymes in leucocytes, urinary copper excretion and organic acids and amino acids in urine. Levels of homovanillic acid (HVA) and vanylmandelic acid (VMA) in CSF and 24 h urine sample are normal. Appropriate studies ruled out immunologic and chronic infectious diseases, and disorders caused by toxic agents.

In cerebrospinal fluid (CSF), cell number, protein content, protein electrophoretic and immunoelectrophoretic patterns, mineral concentrations and levels of HVA and 5-hydroxyindole acetic acid (5HIAA) are normal.

EXAMINATION OF ENERGY METABOLISM

Serum levels of glucose are normal in all patients. Levels of pyruvate and lactate, and the lactate/pyruvate ratios in serum and CSF are shown in Table 2. Serum lactate level shows an intermittent elevation in patient 2. In all patients there is an elevation of CSF pyruvate and lactate levels. Levels of acetoacetate and β-hydroxybutyrate are normal in serum and CSF. 24 h lactate excretion in urine is elevated in patient 1 (148 and 405 μmol/mmol creatinine; normal <100 μmol/mmol creatine) and normal in the other patients. Moderate physical exercise (walking for a few minutes) leads to a marked increase in serum lactate and pyruvate levels in all patients. Only patient 3 shows an abnormal lactate response to oral glucose tolerance loading (1.75 g/kg) as lactate reaches a level of 2300 μmol/l (normal 450-1700 μmol/l), while in all three patients glucose and pyruvate

responses are normal. After intravenous pyruvate loading (500 mg/kg) (Dijkstra et al, 1984), pyruvate response is abnormal in case 2, while lactate response is abnormal in all patients (Fig 4).

Magnetic resonance spectroscopy is not performed in our patients.

Table 2. Lactate and pyruvate levels in serum and cerebrospinal fluid

	Serum			Cerebrospinal fluid		
	Pyruvate*	Lactate*	Ratio L/P	Pyruvate*	Lactate*	Ratio L/P
Patient 1	115-139	1270-1410	9.7-11.9	197-238	3750-5050	18.9-21.2
Patient 2	131-158	1520-2130	10.4-13.5	180	3690	20.5
Patient 3	81-120	810-1160	9.7-10.0	154	3210	20.8
Controls (n=200)	60-155**	460-1720**	up to 15.0	85-132***	1200-1600***	up to 15.0

* μmol/l; ** $P_{2.5}$ - $P_{97.5}$; *** Mean ± 2 SD.

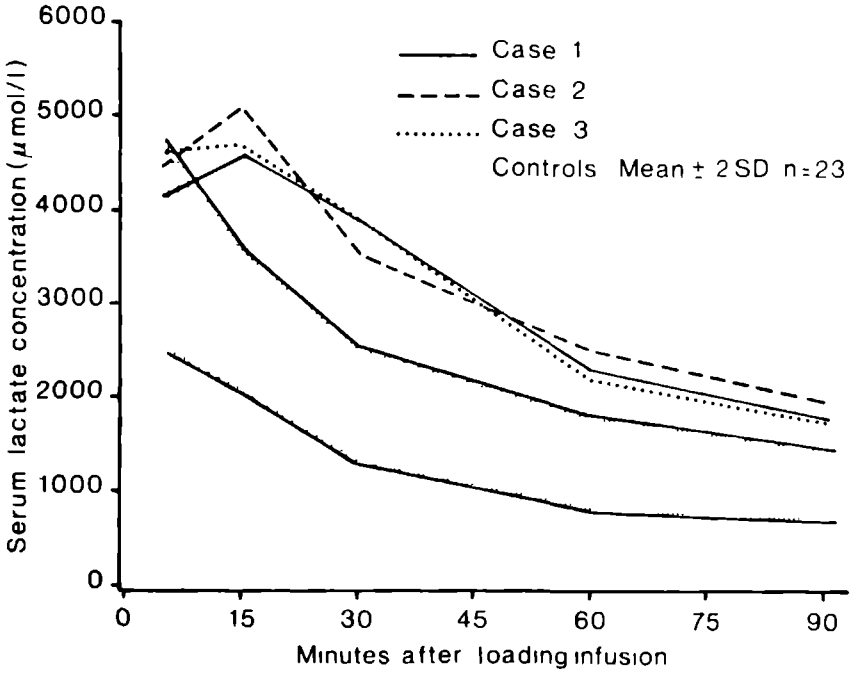

Fig 4. Lactate response to pyruvate loading (500 mg/kg).

BIOCHEMICAL STUDIES

MATERIALS AND METHODS

Enzymatic studies are performed in cultured fibroblasts, and in liver and muscle tissue, obtained at biopsy. Oxidative metabolism in fibroblasts is evaluated by measuring the $^{14}CO_2$ production rate from [1-^{14}C]pyruvate and [2-^{14}C]pyruvate (Willems et al, 1978), and the activities of cytochrome oxidase (Cooperstein and Lazarow, 1951) and citrate synthase (Srere, 1969). Pyruvate carboxylase activity is measured in liver homogenate (Utter and Keech, 1963), using a regenerating system for acetyl-CoA (Henning and Seubert, 1964). Oxidation of [1-^{14}C]pyruvate by liver is measured according to Willems et al (1977) in the presence of 1 mmol/l malate. Pyruvate oxidation rate, and activities of citric acid cycle and respiratory chain are evaluated by measuring $^{14}CO_2$ production from [1-^{14}C]pyruvate and [U-^{14}C]malate in fresh muscle homogenate (Bookelman et al, 1978b). The capacity of muscle to generate adenosine triphosphate (ATP) and creatine phosphate (CrP) is investigated under conditions in which adenylate kinase is inhibited (Ruitenbeek et al, 1981). In muscle homogenate, stored at -70°C, activities of citrate synthase, cytochrome *c* oxidase, succinate: cytochrome *c* oxidoreductase (Fischer et al, 1985), NADH:O_2 oxidoreductase (Fischer et al, 1986b) and NADH:Q_1 oxidoreductase (Fischer et al, 1986b) are measured. Cytochrome content (Bookelman et al, 1978a) is measured in isolated muscle mitochondria in the presence of succinate and cyanide, and subsequently in the presence of antimycin A (1 μg). Protein is assayed according to Lowry et al (1951).

RESULTS

Pyruvate oxidation in fibroblasts is normal in all patients. In liver tissue, pyruvate carboxylase activity is normal in the 2 patients measured (patients 1 and 3). The rate of pyruvate oxidation by liver tissue is normal in patient 1 but decreased in patient 2 (21 nmol $^{14}CO_2$/h/mg protein; controls 45 - 205). Biochemical studies in muscle tissue (see Table 3) reveal that cytochrome levels are normal in all patients. In patient 1, pyruvate oxidation rate and ATP + CrP production rate from pyruvate is mildly reduced when expressed on basis of protein, but when expressed on basis of citrate synthase, the results are normal. So, a clear disturbance is not found. In patients 2 and 3, oxidation rates for pyruvate and malate are decreased, as is ATP + CrP production from pyruvate. In patient 3, the activities of NADH:Q_1 and NADH:O_2 oxidoreductase are reduced.

Table 3. Results of biochemical studies in tissues

	Patient No.			Controls	
	1	2	3	Range	No
Oxidation (600 g supernatant)					
[1-^{14}C]pyruvate + malate	212[1]	123	69	273-705	20
[1-^{14}C]pyruvate + carnitine	356[1]	151	103	266-941	20
[U-^{14}C]malate + pyruvate + malonate	340[1]	150	69	320-996	20
[U-^{14}C]malate + acetylcarnitine + malonate	351[1]	156	69	317-1155	20
[U-^{14}C]malate + acetylcarnitine + arsenite	291[1]	91	34	198-274	19
ATP Metabolism (600 g supernatant)					
ATP + CrP production from pyruvate	2921[2]	1214	496	3354-9993	20
Enzyme activities (600 g supernatant)					
Citrate synthase	18[3]		57	27-77	18
Cytochrome *c* oxidase	191[3]	100	166	73-284	39
Succinate cytochrome *c* oxidoreductase			22[4]	22-84	18
NADH:O_2 oxidoreductase			8.5[5]	14-45	12
NADH Q_1 oxidoreductase			5.0[5]	8.7-25.3	11
Cytochromes (isolated mitochondria)					
Cytochrome aa_3	369[6]	647	486	271-585	14
Cytochrome *b*	382[6]	405	488	317-511	14
Cytochrome $c + c_1$	534[6]	482	531	365-923	14
Cytochrome *c* oxidase	2.60[7]	2 70	4 26	1 29-3 43	16

[1] nmol $^{14}CO_2$/h/mg protein, [2] nmol/h/mg protein, [3] mU/mg protein, [4] nmol cytochrome *c* reduced/min/mg protein, [5] nmol NADH oxidized/min/mg protein; [6] pmol/mg protein; [7] U/mg protein.

HISTOPATHOLOGIC STUDIES

In all patients, histochemical studies of quadriceps muscle tissue, obtained by biopsy, show no abnormalities. No 'ragged-red fibres' are noted in the trichromic stained sections, and no Sudan-black positive droplets are seen. Immunohistochemical studies of cytochrome oxidase reveal no abnormalities. Ultrastructural studies also show no abnormalities.

THERAPY

Therapy with L-Dopa and benseraside (Madopar®) did not result in any improvement in our patients.

DISCUSSION

We report three sporadic patients with a progressive degenerative neurological disorder. The clinical picture is dominated by severe hypokinesia and rigidity, although in two patients slight athetotic or dystonic movements are seen. The association of dystonia and parkinsonism in untreated patients, as is seen in our patients, albeit uncommon, has been reported more often (Katchen and Duvoisin, 1986; Lewitt et al, 1986). Our patients show frequent sighing and abrupt changes in respiratory and cardiac rate. Pyramidal and cerebellar signs are present in all three and ocular signs in two patients. Their clinical status deteriorates markedly on intercurrent infections, followed by slow recovery. Cognitive functioning is normal and there are no epileptic manifestations. Neurophysiological studies reveal no abnormalities. Cerebral CT and MRI show bilateral putaminal lesions in patients 1 and 2, lesions of the head of the right nucleus caudatus in patient 1, and bilateral lesions of the internal capsule in patient 3. Chemical investigations of serum, urine and CSF reveal as most conspicuous findings an elevation of CSF lactate, pyruvate and lactate-pyruvate ratio, a marked increase in serum lactate and pyruvate levels after a moderate physical exercise, and an abnormal lactate response to pyruvate loading in all patients. These abnormalities point to a disturbance of pyruvate metabolism. In liver and muscle tissue, no histochemical or ultrastructural abnormalities are seen, but biochemical studies reveal a NADH dehydrogenase deficiency in patient 3 (Table 3). A disturbed functioning of the respiratory chain could be a possible cause for the reduced pyruvate oxidation rate found in patient 2. As the cytochrome content is normal, the defect must be localised at the level of the NADH dehydrogenase or at the level of coenzyme Q (Fischer et al, 1986a). At the time of the muscle biopsy, enzyme assays for this part of the respiratory chain were not yet available. In patient 1 no biochemical abnormalities are found in muscle tissue. A defect of oxidative metabolism restricted to brain is possible in this patient.

Our patients are suffering from a mitochondrial encephalomyopathy according to the definition of Shapira et al (1977).

The differential diagnosis of CT scan abnormalities similar to the putaminal lesions seen in our patients and in other patients with Leigh syndrome (Hall and Gardner-Medwin, 1978) is considerable. Moreover, the differential diagnosis is not necessarily restricted to parkinson-like hypokinetic-rigid syndromes. Depending on the kind and extension of lesions in various parts of the striopallidum, the resulting functional disorders may be associated with a variable symptomatology of postural disorders and involuntary movements, that can manifest in kaleidoscopic symptoms, while in other cases the pathological lesions are not necessarily

associated with any clinical symptoms at all. This also holds true for Leigh syndrome (Montpetit et al, 1971; Sipe, 1973). Bilateral parkinsonian rigid hypokinesia and dysarthria, as seen in our patients, is called the pseudobulbar syndrome (Denny-Brown, 1962). Hypokinesia can be regarded as the primary symptom of nigrostriatal failure (Denny-Brown, 1962; Marsden, 1982), while rigidity can be considered as caused by pathological changes in the putamen and the release of pallidofugal discharge (Martin, 1965). The pseudobulbar syndrome may be caused by infarcts (Denny-Brown, 1962), which are sometimes visible on CT scan (Tolosa and Santamaria, 1984; Friedman et al, 1986), by methanol (Bourrat et al, 1986) or by carbon monoxide intoxication (Klawans et al, 1982). None of these causes seem to be present in our patients.

Putaminal CT lesions are associated with dystonia in the patients of Burton et al (1984), in patients with familial dystonia with optic atrophy (Marsden et al, 1986; Novotny et al, 1986) and in some cases of infantile bilateral striatal necrosis (Goutières and Aicardi, 1982). The clinical data of the two children described by Burton et al (1984) are compatible with Leigh syndrome. Familial dystonia with optic atrophy is excluded in our patients because of the exclusive dystonic syndrome in such patients. Infantile bilateral striatal necrosis may also be associated with hypokinesia, rigidity or a mixture of extrapyramidal symptoms (Miyoshi et al, 1968; Roessmann and Schwartz, 1973; Roytta et al, 1981). Goutières and Aicardi (1982) divide the syndrome of infantile bilateral striatal necrosis into three subgroups: 1) those of definite or probable Leigh syndrome, 2) cases of familial degeneration of the striatum with an insidious onset and a slow progressive course, and 3) cases which present with abrupt neurological dysfunction following an acute systemic illness. The distinguishing feature with our patients is the presence of mental retardation and the absence of metabolic features of Leigh syndrome. Our patients show features of both the first and the third subgroup: metabolic abnormalities strongly suggest Leigh syndrome, and there is a deterioration during systemic illnesses. Because the latter phenomenon is frequently seen in patients with Leigh syndrome (Table 4), the first and the third subgroup of Goutières and Aicardi may be identical. Wilson disease (Williams and Walshe, 1981) and anoxia (Aicardi et al, 1985), which also may cause putaminal CT abnormalities and extrapyramidal symptoms, have been excluded in our patients.

Hallervorden-Spatz syndrome (Dooling et al, 1974) and juvenile Huntington chorea (Jongen et al, 1980) are always characterized by a rapidly progressive mental deterioration and, to our knowledge, there are no CT abnormalities in the putamen. Moreover, in Huntington chorea, there is a positive family history. In patients with Hallervorden-Spatz syndrome (2 own patients, unpublished data) and juvenile Huntington chorea (Jongen

et al, 1980) normal CSF lactate and pyruvate levels are found. None of our patients has a history of use of phenothiazines or butyrophenones, or drug abuse.

Signs and symptoms of our patients are compatible with Leigh syndrome. In Table 4, signs and symptoms of our patients are compared with data from a literature study of 173 patients with proven Leigh syndrome, that show ocular signs, pyramidal and respiratory signs, and feeding problems in more than half of the cases.

CT and/or MRI abnormalities bilaterally in the basal ganglia, most striking in the putamina and often associated with hemispheral or brainstem lesions have been reported in a number of proven and suspected Leigh patients (Hall and Gardner-Medwin, 1978; Ollivier and Diebler, 1980; Schwartz and Hutchinson, 1981, with additional remark of Weisberg, 1982; Chi et al, 1981; Burton et al, 1984; Onuma et al, 1986; Koch et al, 1985, 1986; Davis et al, 1987). In none of these patients, however, the movement disorder is dominated by a parkinson-like hypokinetic rigid syndrome as seen in our patients. MRI is superior to CT in that it may show abnormalities not yet apparent in CT (Geyer et al, 1986; Koch et al, 1986; Onuma et al, 1986). In the putaminal regions of patients 1 and 2 the T_2 relaxation times are significantly prolonged (Table 1), corresponding with abnormal CT and MRI images of those regions. The T_1 values are abnormal only in patient 2. The calculated T_1 and T_2 values of the thalamus (Table 1) show normal T_2 values and prolonged T_1 values in patients 1 and 2 without any abnormality in CT or MRI images of this area. It might, therefore, be useful in suspected Leigh patients to calculate the T_1 and T_2 values of clinically relevant areas even if MRI pictures are not abnormal. However, it is not necessary that basal ganglia abnormalities can be visualized by

Table 4. Signs and symptoms of our patients compared with signs and symptoms of 173 proven Leigh patients (age at onset between 0 to 16 years) from the literature and with a subgroup of 24 juvenile Leigh patients (age at onset between 4 to 16 years) from the literature

	Patient No.			Patients from literature (n=173)	Juvenile patients from literature (n=24)
	1	2	3		
Feeding problems	+	-	-	55%	42%
Repiratory signs and symptoms	+	+	+	69%	54%
Cardiac signs and symptoms	+	+	+	18%	25%
Ocular signs	+	+	+	78%	79%
Pyramidal signs	+	+	+	61%	88%
Extrapyramidal signs	+	+	+	24%	46%
- hypokinesia-rigidity	+	+	+	9%	25%
- dystonia	+	-	-	6%	13%
Cerebellar signs	+	+	+	39%	54%
Exercise intolerance	+	+	+	47%	50%
Deterioration on infections	+	+	+	39%	42%

CT or MRI in Leigh syndrome, as we found no evidence of such abnormalities in four other cases of proven Leigh syndrome.

Leigh syndrome has been associated with various types of mitochondrial deficiencies. Deficiencies of pyruvate carboxylase (Hommes et al, 1968), pyruvate dehydrogenase complex (Farmer et al, 1973), cytochrome *c* oxidase (Willems et al, 1977), NADH dehydrogenase (Van Erven et al, 1985), and a disturbance in the activation of the pyruvate dehydrogenase complex (DeVivo et al, 1979) have been reported in Leigh syndrome.

In conclusion, signs and symptoms, the association with a disturbance of pyruvate metabolism and the bilateral putaminal lesions on CT and MRI make the clinical diagnosis of Leigh syndrome in these patients highly probable.

We would like to stress that in children with a disorder presenting with hypokinesia and rigidity, especially if accompanied with basal ganglia lesions on CT or MRI, mitochondrial energy metabolism should be investigated in order to evaluate the possibility of Leigh syndrome. In these patients, lactate and pyruvate levels of serum and CSF should be measured. In some cases a pyruvate loading test can also provide useful information (Van Erven et al, 1987).

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CHAPTER IV

NEUROPHYSIOLOGICAL STUDIES IN THE LEIGH SYNDROME

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ABSTRACT

We performed neurophysiological studies in 12 patients with the Leigh syndrome (6 pathologically confirmed and 6 clinically diagnosed). The results are compared with data derived from a literature survey of 173 Leigh syndrome patients. We found no positive contribution of neurophysiological studies towards the diagnosis of the Leigh syndrome.

INTRODUCTION

The Leigh syndrome (1) is one of the so-called mitochondrial encephalomyopathies (2). The latter are a group of degenerative neurological disorders associated with disturbances in mitochondrial energy metabolism and/or morphologically abnormal mitochondria. A definite diagnosis can only be made on post mortem examination of the central nervous system. The pathological abnormalities are well-defined and consist of symmetrical, bilateral, sharply demarcated areas of spongy degeneration and demyelination, characterized by vascular, endothelial and glial proliferation, with relative sparing of the neurons (3). The highest incidence of involvement is shown by the brainstem tegmentum, basal ganglia, spinal cord and cerebellum (4). No specific diagnostic tests are available. Apart from the diagnosis of a disturbance of pyruvate metabolism, the value of chemical and biochemical investigations lies in the exclusion of other metabolic diseases, deficiencies, intoxications, and endocrinologic and immunologic disorders.

Neuroradiological abnormalities are not specific: the bilateral hypodensities in the basal ganglia region seen on cerebral CT scanning (5) are also found in Parkinson disease, carbon monoxide poisoning and Wilson disease.

In order to investigate the diagnostic value of neurophysiological studies in this category of patients, we performed electroencephalography (EEG), electromyography (EMG) and nerve conduction velocity studies, and measured evoked potentials (visual evoked potentials, VEP; brainstem auditory evoked potentials, BAEP; short latency somatosensory evoked potentials, SSEP) in 12 patients with the Leigh syndrome (6 pathologically confirmed and 6 clinically diagnosed).

PATIENTS AND METHODS

PATIENT SELECTION

We studied 12 patients. In patients 1 and 3, and a sib of patient 2, the clinical diagnosis of the Leigh syndrome was confirmed on pathological examination. Patients 4, 5 and 6 are sibs of patient 3. So, the diagnosis is proven in patients 1 to 6. For patients 7 to 12, who suffer from mitochondrial encephalomyopathies, we made a clinical diagnosis of 'most probable Leigh syndrome' on the basis of a combination of three parameters: clinical signs and symptoms, autosomal recessive mode of inheritance, and disturbance of pyruvate metabolism. The signs and symptoms in our patients are summarized in Table 1. They are compared

Table 1. Summary of the clinical data of our patients* compared with the percentages derived from a literature study of 173 Leigh syndrome patients

Patient No.	1	2	3	4	5	6	7	8	9	10	11	12	Total (%)	Literature (%)
Age (yrs)	17†	8	17†	24	23	20	17	13	7	4	9	14		
Age at onset (yrs)	4	1	6	3	9	4	8	9	1	2	6	0		
Sex	F	F	M	M	F	F	M	M	M	M	M	F		
Height	N	P_{10}	N	N	N	N	$P_{2.5}$	P_5	N	P_5	N	N		
Weight	N	P_{10}	N	N	N	N	$P_{2.5}$	$P_{2.5}$	N	P_5	N	N		
Skull circumference	N	P_{10}	N	N	N	N	P_{10}	N	N	N	N	N		
Feeding problems	–	+	–	–	–	–	–	–	+	+	–	–	25	55
Respiratory signs and symptoms	+	+	+	+	+	+	+	–	+	+	+	+	92	69
Cardiac signs and symptoms	–	+	+	+	+	+	+	+	+	+	–	+	83	18
Ocular signs	+	–	–	–	+	–	+	+	+	+	+	+	58	78
Pyramidal signs	+	+	+	+	+	+	+	+	+	+	+	+	100	61
Extrapyramidal signs	–	–	–	+	+	–	+	+	+	–	–	+	50	24
Cerebellar signs	–	+	+	+	+	+	+	+	+	+	+	+	83	39
Hypotonia	–	–	–	+	+	+	–	–	–	+	–	+	43	69
Seizures	–	–	–	–	–	–	–	–	–	–	–	+	8	36
Exercise intolerance	–	+	–	–	–	–	+	+	+	+	+	+	58	47
Decompensation on infection	+	+	+	+	+	+	+	+	+	+	+	+	100	39
Mental retardation/deterioration	–	–	–	+	±	–	–	–	±	–	–	+	43	37

* Patients 1 to 6: diagnosis confirmed; patients 7 to 12: most probable Leigh symdrome.
† = deceased; M = male; F = female; P = percentile; N = normal; + = present; – = absent; ± = mild.

with the results of a literature study of 173 proven Leigh syndrome patients, with a median age at onset of 0.83 years and a median age at death of 2.08 years. The differences in the occurrences of signs and symptoms in our patients can be explained by the differences in age and the chronic protracted course of the disease in all our patients. In all patients there were strong indications of a disturbance of pyruvate metabolism: CSF pyruvate and/or lactate levels were elevated in all patients but one (patient 11), and the pyruvate loading test (6) was disturbed in all patients. Biochemical studies of tissues were performed in 9 patients and defects in mitochondrial energy metabolism were found in 7 (Table 2).

METHODS

EEGs were recorded on a 21-channel Siemens Mingograph, with silver-chloride cup electrodes placed according to the international 10-20 system. EEG recordings were made under standard conditions: 30 minutes of wakeful rest with closed eyes, and then photic stimulation and two periods of hyperventilation for 3 minutes. The EEG was considered non-disturbed when there was age-adequate dominant activity with little dispersion, good spatial differentiation, good reaction at eye opening, normal quantities of variants and other activities, and no spikes or instability of activities. A Medelec MS 6 was used for EMG and nerve conduction velocity studies. EMG was performed with DISA concentric needle-electrodes. Nerve conduction velocities were recorded with surface silver-chloride disc electrodes. In all patients, nerve conduction velocity studies of the peroneal and sural nerves, H-reflexes over the soleus muscles and electromyography of the distal and proximal muscles of the legs were performed. Evoked potentials were recorded with a Nicolet Pathfinder II, using the protocols summarized in Table 3. For normal values of evoked potentials, see Colon et al (7,8).

RESULTS

In 5 patients there were EEG abnormalities. Four had slight diffuse nonspecific abnormalities, such as dysrhythmia and slight slowing. One patient showed focal (right temporal) 2½-3/s high amplitude discharges with polyspike bursts, and generalized paroxysmal epileptic activity.

The results of EMG and nerve conduction velocity studies were normal in all patients, except in sibs 3, 4 and 5. These 3 patients showed motor and sensory neuropathy of the legs beginning with an increase in distal motor and sensory latencies, and at a later stage, a decrease in motor

Table 2. Results of biochemical studies in tissues

	Fibroblasts	Liver	Muscle			
Patient No.	Pyruvate oxidation	Pyruvate carboxylase	Pyruvate oxidation	Pyruvate, malate oxidation	ATP production	Respiratory chain defect
1	N	N	↓	↓	↓	NADH dehydrogenase deficiency
2	–	N	↓	↓	–	NADH dehydrogenase deficiency
3	N	N	–	N	N	N
7	N	N	–	↓	↓	NADH dehydrogenase deficiency
8	N	–	↓	↓	↓	N
9	N	N	–	N	N	N
10	N	N	↓	↓	↓	no localized defect
11	N	N	↓	↓	↓	no localized defect
12	–	–	–	↓	↓	cytochrome oxidase deficiency

N = normal; ↓ = decreased; – = not measured.

Table 3. Evoked potential protocols

	VEP *(flash)* *(eyes open, wakeful rest)*	BAEP *(eyes open, wakeful rest)*	SSEP *(short latency)* *(eyes open, wakeful rest)*
Stimulating	OD and OS	AD and AS	Median nerve (wrist)
Stimulation	Flash 0.2 ms (0.1 J at 30 cm) 0.30/s Random	Click 0.1 ms 70 dB HL (80 dB SPL) 11.1/s Rarefaction Regular	Electrical 0.1 ms Twitch level 3.1/s Regular
Band pass	1 – 500 Hz	150 – 3000 Hz	5 – 1000 Hz
Analysis time	800 ms*	16 ms*	200 ms*
Sensitivity	100 or 200 μV	20 μV	200 μV
Number of stimuli	100 (twice)	1000 (twice)	1000 (twice)
Electrodes	0_z – A_2	preauricular R – C_z preauricular L – C_z	SC_5, C_3' or C_4' – A ipsi

* 25% prestimulus interval incorporated into indicated analysis time.

nerve conduction velocities (peroneal nerves 30 m/s). In the arms, they showed distal motor and sensory neuropathy of a much lesser degree. EMG in these patients showed high amplitude, polyphasic motor unit potentials of long duration: a picture compatible with a chronic neurogenic lesion.

Sural nerve biopsy specimens from patients 3 and 5 showed a chronic process of axonal degeneration, with a near total loss of myelinated and also, but to a lesser extent, unmyelinated fibers. Incidental Wallerian degeneration was seen, but no segmental abnormalities.

VEP were recorded in 9 patients and were normal in all cases.

BAEP were normal in all 10 patients studied.

SSEP were found to be normal in 7 patients and not elicitable in 1 patient (polyneuropathy), and were not investigated in the other 4 patients. An example of evoked potential recordings in a patient with proven Leigh syndrome (patient 2) is presented in Fig 1.

The results are summarized in Table 4.

DISCUSSION

We performed clinical neurophysiological studies (EEG, EMG and nerve conduction velocities, VEP, BAEP and SSEP) in 12 patients with mitochondrial encephalomyopathy of the Leigh type. 40% showed EEG

Table 4. Summary of results of electrophysiological studies in 12 patients with the Leigh syndrome

Patient No.	EEG	EMG/nerve conduction	VEP	BAEP	SSEP
1†	slight diffuse slowing	N	-	-	-
2	N	N	N	N	N
3†	N	polyneuropathy	-	-	-
4	slight diffuse nonspecific disturbance	polyneuropathy	N	N	not elicitable
5	slight diffuse slowing	polyneuropathy	N	N	-
6	N	N	N	N	-
7	N	N	N	N	N
8	N	N	-	N	N
9	N	N	N	N	N
10	N	N	N	N	N
11	slight diffuse nonspecific disturbance	N	N	N	N
12	focal (right temporal region) and generalized epileptic activity	N	N	N	N

N = normal; - = not performed; † = deceased.

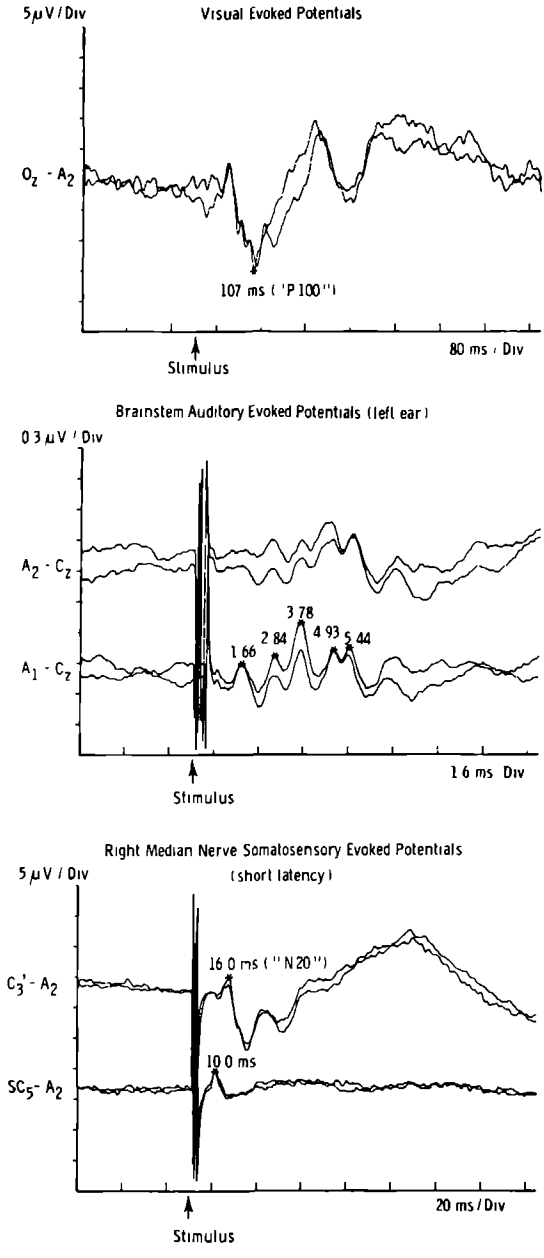

Fig 1. Recordings of normal VEP, BAEP and median nerve SSEP in patient 2. The protocols are summarized in Table 3. For normal values, see Colon et al (7,8,).

abnormalities, all of which involved nonspecific phenomena, and axonal polyneuropathy was found in three sibs, in one of whom no median nerve SSEP were elicitable. The other studies showed normal results.

173 pathologically proven Leigh syndrome patients in the literature were surveyed (10)(Table 5). For 50% (n=87) the EEG findings and for 18% (n=31) the EMG findings were mentioned. VEP were recorded in 2% (n=4) and BAEP in 0.5% (n=1). We found no mention of SSEP recording in the Leigh syndrome.

EEG was unremarkable in 31 patients, and showed epileptic discharges in 17, and diffuse or focal abnormalities, of which diffuse slowing was the most frequent (n=29), in 43. In 8 patients, EEG was normal initially and became abnormal during the course of the disease. EMG was normal in 17 patients. Two patients showed denervation and myopathic features were found in 3. Nerve conduction velocities were normal in 13 patients and reduced in 15. Distinction between motor and sensory nerve conduction was only seldom made. VEP were normal in 2 patients and disturbed/non-elicitable in 2. BAEP were disturbed in 1 patient. Three patients mentioned in a report by Hecox et al (9) were not included in our series of 173 patients, because a description of the patients is lacking. Normal BAEP were recorded in 1 patient, tested at age 3 months, and abnormalities

Table 5. Electrophysiological studies in 173 Leigh syndrome patients in the literature

EEG (n=87):	Normal			31
	Diffuse slowing			29
	Focal slowing			6
	Increase of amplitude			4
	Dysrhythmia			2
	Epileptic manifestations			17
	- generalized spikes		3	
	- paroxysms		4	
	- focal spikes		4	
	- spike and wave complexes		4	
	- hypsarhythmia		2	
EMG (n=31):	Electromyography:	Normal		17
		Denervation		2
		Myopathy		3
	Nerve conduction velocities:	Normal		13
		Reduced:	motor+sensory	11
			motor	3
			sensory	1
VEP (n=4):	Normal			2
	Not elicitable over right occipital cortex			1
	Not elicitable			1
BAEP (n=1).	Right ear normal; left ear: not elicitable			(1)

of interwave intervals were present in responses obtained from 2 patients at age 1 and 6 years respectively.

Many difficulties arose as to the interpretation of the results of the literature study. Descriptions of findings are often inadequate, incomplete and/or unhomogeneous. The median age at death of the 173 patients studied was 2.08 years. Interpretation of EEG is notably difficult in these often very young patients. Furthermore, the EEGs were often recorded in relative inactive phases of the disease. In a considerable number of patients there was acute onset or aggravation of symptoms, leading to death in a few days. In such cases, hardly any EEGs were recorded, but there was often mention of epileptic seizures or status epilepticus. Most of the patient reports appeared before 1972; this could explain the paucity of EMG and evoked potential recordings.

Although the findings in our patients, a subgroup with a relatively late onset and chronic progressive course of the disease, cannot be compared with those in the patients mentioned in the literature, they support the concepts that EEG abnormalities are nonspecific, and that EEG does not contribute to the diagnosis.

EMG and nerve conduction disturbances are rare in our patients (3 sibs), contrary in the cases of the patients mentioned in the literature (Table 5).

In our patients, the afferent sensory pathways are clinically intact and the symptomatology arises from efferent structures (Table 1). The intactness of the central sensory pathways explains the lack of evoked potential abnormalities.

From our results we conclude that neurophysiological studies did not contribute to the diagnosis of the Leigh syndrome in our patients. Their relevance lies rather in the elimination of other differential diagnostic possibilities.

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CHAPTER V

INTRAVENOUS PYRUVATE LOADING TEST IN LEIGH SYNDROME

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ABSTRACT

Diagnosis of defective pyruvate metabolism can present difficulties in clinical practice. In search of a diagnostic procedure that can give a clear indication of a disturbance of pyruvate metabolism, we have developed an intravenous pyruvate loading test. The loading test was applied to 9 patients with Leigh syndrome. Results and characteristics are described. The test proved to be a sensitive procedure to detect disturbances in pyruvate oxidation. The intravenous pyruvate loading test can be a useful tool in the diagnosis of mitochondrial (encephalo)myopathies.

INTRODUCTION

The variability in clinical presentation and the lack of specific laboratory tests make defects of pyruvate metabolism often a difficult diagnostic problem in clinical practice. In the screening of these patients assessment of pyruvate and lactate levels of serum under standard conditions, upon fasting, after exercise, and after oral glucose loading, play an important role, as well as pyruvate and lactate levels of cerebrospinal fluid (CSF) and 24-h urine lactate excretion. Often, however, these relatively simple diagnostic procedures provide insufficient information. Sometimes even, all the results are normal but a defect in oxidative metabolism is found on biochemical studies in tissues nonetheless.

In recent years, defects of oxidative metabolism have been described in a number of patients with Leigh syndrome (Hommes et al, 1968; Farmer et al, 1973; Willems et al, 1977; Van Erven et al, 1985). In search of a procedure that can give direct indication of a disturbance of pyruvate metabolism - or in a broader sense of oxidative metabolism - we performed an intravenous pyruvate loading test (Dijkstra et al, 1984) on 9 patients with Leigh syndrome (3 confirmed, 6 most probable), with elevation of lactate and pyruvate levels in body fluids and/or an established defect of pyruvate metabolism in liver or muscle tissue.

CRITERIA OF PATIENT SELECTION

The diagnosis of subacute necrotizing encephalomyelopathy (SNE) or Leigh syndrome rests on pathologic findings. Since no biopsy of afflicted central nervous system (CNS) areas is possible and since there is no specific test available, durante vitam only the probability of Leigh syndrome can be assessed after endocrinologic, immunologic and chronic infectious diseases, deficiencies, disorders caused by toxic agents, degenerative and other metabolic diseases have been ruled out by appropriate studies. Based on own experience and on the literature, it is, in our opinion, possible to come to a clinical diagnosis of 'most probable' Leigh syndrome when the following criteria are met:

(1) Signs and symptoms that arise mainly from the brain regions that show the highest incidence of the typical lesions on pathologic examination: midbrain, hindbrain and spinal cord, basal ganglia, cerebellum and optical system (Montpetit et al, 1971; Pincus, 1972),

(2) Disturbance of pyruvate metabolism (Hommes et al, 1968; Farmer et al, 1973; Willems et al, 1977; Van Erven et al, 1985). Pyruvate metabolism is considered disturbed when pyruvate and/or lactate levels of CSF, serum

or urine are elevated, or when a disturbed pyruvate oxidation is found in biochemical studies of tissue samples,

(3) Autosomal recessive mode of inheritance (McKusick, 1978).

We studied 9 patients with Leigh syndrome. In one patient the clinical diagnosis was confirmed on pathologic examination (patient 1, Van Erven et al, 1985). Two patients had sibs who died of pathologically confirmed Leigh syndrome (patients 2, Van Erven et al, 1986, and 3). The other 6 patients matched the criteria listed above and were classified 'most probable' Leigh syndrome (patients 4-9). The mean age at which the pyruvate loading test was performed was 13 years (range, 5-17 years). Clinical data of our patients are presented in Table 1.

Extensive routine and specific chemical and biochemical investigations yielded normal results in all patients. EEG showed a generalized epileptic predisposition in patient 9. Nerve conduction velocity studies revealed an axonal polyneuropathy in patients 3 and 8, and cerebral CT scan showed bilateral hypodensities in the basal ganglia area in patients 4, 5 and 6. Histochemical and ultrastructural studies in muscle tissue were performed in all patients. No Sudan black-positive droplets were seen in histochemical studies. 'Ragged-red' fibers were seen in the trichromic-stained section in patient 9. Ultrastructural studies revealed no mitochondrial abnormalities.

Table 1. Summary of clinical data of patients
Patients 1, 2 and 3: diagnosis confirmed, Patients 4 to 9 most probable Leigh syndrome

Patient No	1	2	3	4	5	6	7	8	9
Age (yrs)	17†	8	24	17	13	7	4	9	14
Age at onset (yrs)	4	1½	3	8	9	1½	2	6	0
Sex	F	F	M	M	M	M	M	M	F
Height	N	P_{10}	N	$P_{2.5}$	P_5	N	P_5	N	N
Weight	N	P_{10}	N	$P_{2.5}$	$P_{2.5}$	N	P_5	N	N
Skull circumference	N	P_{10}	N	P_{10}	N	N	N	N	N
Feeding problems	–	+	–	–	–	+	+	–	–
Respiratory signs and symptoms	+	+	+	+	–	+	+	+	+
Cardiac signs and symptoms	–	+	+	+	+	+	+	–	+
Ocular signs	+	–	–	+	+	+	+	+	+
Pyramidal signs	+	+	+	+	+	+	+	+	+
Extrapyramidal signs	–	–	+	+	+	+	–	–	+
Cerebellar signs	–	+	+	+	+	+	+	+	+
Hypotonia	–	–	+	–	–	–	+	–	+
Seizures	–	–	–	–	–	–	–	–	+
Exercise intolerance	–	+	–	+	+	+	+	+	+
Decompensations on infections	+	+	+	+	+	+	+	+	+
Mental retardation/deterioration	–	–	+	–	–	±	–	–	+

† = deceased, M = male, F = female; P= percentile, N = normal, + = present; – = absent; ± = mild.

METHODS

Pyruvate metabolism was assessed by determination of the levels of pyruvate and lactate, and their ratios in serum and CSF, and by measuring 24-h lactate excretion in urine. All patients underwent an oral glucose tolerance test (GTT) (1.75 g/kg) with concomitant measuring of lactate and pyruvate.

PYRUVATE LOADING TEST

Informed consent was obtained from all patients, or their parents, and controls. The pyruvate loading test was performed after an overnight fast: 500 mg sodium pyruvate/kg body weight was administered in a period of 10 min using a 0.91 M sodium-pyruvate solution. Antecubital venous blood samples were drawn from the other arm before and at 5, 15, 30, 60 and 90 min after infusion. Glucose, pyruvate, lactate and alanine were measured in all samples. Nine healthy adult volunteers (mean age, 29 years; range, 20-43 years) and 14 children (mean age, 10 years; range, 4-17 years) formed the controls. The control children were suffering from progressive heredodegenerative neurologic disorders (e.g. spinal muscular atrophy, lateral sclerosis, progressive polyneuropathy and heredoataxia). In all children a disturbance of pyruvate metabolism was included in the differential diagnosis. Pyruvate and lactate levels were normal in body fluids and also after oral glucose loading. Biopsies of muscle and sural nerve were performed in all children, and morphologic and biochemical investigations revealed no abnormalities of pyruvate metabolism. Adults and children showed the same response after pyruvate loading. Some subjects both in the control and in the patient group had slight complaints, e.g. tachycardia, headache, trembling, nausea, perspiration and fatigue during the first 15 min after pyruvate infusion.

BIOCHEMICAL STUDIES IN TISSUES

Pyruvate oxidation and citric acid cycle activity in fibroblasts were determined by measuring $^{14}CO_2$ production from [1-^{14}C]pyruvate and [2-^{14}C]pyruvate (Willems et al, 1978). Pyruvate carboxylase activity was measured in liver tissue homogenate (Utter and Keech, 1963). Pyruvate oxidation in liver tissue was determined by measuring $^{14}CO_2$ production from [1-^{14}C]pyruvate according to Willems et al (1977). Pyruvate oxidation rate and activities of citric acid cycle and respiratory chain were evaluated in muscle homogenate by measuring $^{14}CO_2$ production from [1-^{14}C]pyruvate and [U-^{14}C]malate (Bookelman et al, 1978). ATP production was measured

according to Ruitenbeek et al (1981). The activities of the enzymes cytochrome *c* oxidase, citrate synthase, succinate-cytochrome *c* oxidoreductase and carnitine palmitoyltransferase were measured in liver and/or muscle tissue (Cooperstein and Lazarow, 1951; Srere, 1969; Scholte et al, 1979; Fischer et al, 1985).

STATISTICAL ANALYSIS

The logarithm of the pyruvate and lactate concentrations was used in the statistical calculations. This allows the use of standard statistical methods based on assumptions regarding homogeneity of variance and normality of distribution. Student's *t*-test for the unpaired case is used to judge, on each sampling time after infusion, the difference in the mean relative value of the serum concentration between the control group and the Leigh patient group. In order to determine to what degree the two groups can be discriminated by means of the pyruvate and lactate values, we used the Hotelling T^2-test, followed by a classification technique based on the linear discriminant function originally introduced by Fischer (see Morrison, 1967). For diagnostic aids we constructed a tolerance region for the control group on a combination of one response parameter of the lactate response and one parameter of the pyruvate response curve.

RESULTS

Lactate and pyruvate contents of body fluids are shown in Table 2. Serum pyruvate levels were normal or nearly normal in all patients. Serum lactate levels showed an intermittent increase in patients 1 and 5. CSF pyruvate and lactate levels were increased in all patients but one (patient 8). Lactate-pyruvate ratio in CSF was normal in patients 8 and 9. 24-Hour lactate excretion in urine was clearly elevated in patients 6 and 7, and showed an intermittent elevation in patient 9. After oral glucose loading, lactate and pyruvate levels were elevated in patients 1 and 4.

PYRUVATE LOADING TEST

Glucose levels did not change significantly after pyruvate infusion. Alanine levels reached their maximum at 5 min after pyruvate infusion and normalized at 90 min. Mean alanine increase (about 200 μmol/l) did not differ between controls and patients, and the response was similar in both groups. Relative serum pyruvate levels of the patient group were signi-

Table 2. Lactate and pyruvate levels in serum and cerebrospinal fluid, lactate levels in 24-hour urine, and results of glucose tolerance tests of patients and controls

Patient No.	Serum		Cerebrospinal fluid			24-hour lactate[b] excretion in urine	GTT + lactate and pyruvate
	Pyruvate[a]	Lactate[a]	Pyruvate[a]	Lactate[a]	Ratio L/P		
1	152-156	1600-2330	143-198	2520-4470	17.6-22.6	-	↑ L + P
2	118-126	1280-1700	90-153	1520-1830	12.0-16.9	52-91	N
3	113-156	925-1540	201	3760	18.7	49	N
4	81-120	810-1160	154	3210	20.8	36	↑ L + P
5	131-158	1520-2130	180	3690	20 5	60-70	N
6	115-139	1270-1410	197-238	3750-5050	18 9-21.2	148-405	-
7	108-137	1000-1220	144-216	3120-3780	17.5-21.7	158-669	N
8	90	950	98	1400	13.3	31	-
9	94-148	1380-1460	222	1900	8.6	30-131	N
Controls	60-155[c]	460-1720[c]	85-132[d]	1200-1600[d]	up to 15.0	< 100	

N = normal; ↑ = increased; - = not performed
[a] μmol/l; [b] μmol/mmol creatinine; [c] $P_{2.5} - P_{97.5}$; [d] mean ± 2 SD.

ficantly higher at 5, 15, 30, 60 and 90 min after infusion compared with the control group (Table 3). The shape of the pyruvate curves was similar in both groups, unlike the shape of the lactate curves, where the patient group showed a slower rise and reached a peak-level at 15 min after infusion (t_{15}) compared with a peak-level at 5 min (t_5) in the control group. The peak-level reached similar values in patients and in controls. Between 15 and 90 min, relative lactate levels were significantly higher than in the control group (Table 3). Lactate-pyruvate ratios in the patient group were significantly lower than in the control group at t_5 and t_{15}.

Results of discriminant analysis are presented in Table 4. From these data it is clear that Leigh patients respond quite different from a control group with respect to the pyruvate loading test. In order to make this finding suitable for diagnostic purposes we had to construct some region

Table 3. Mean pre- and postinfusion levels ± SD of pyruvate and lactate (μmol/l), their mean relative serum levels, and lactate-pyruvate ratios

Time (min)	Pre- and postinfusion levels		Mean relative serum levels		
	Controls (n=23)	Leigh patients (n=9)	Controls	Leigh patients	P^a
Pyruvate					
Preinfusion	121 ± 71	126 ± 26	1.	1	
5	1991 ± 763	5323 ± 2065	20 7	44 1	0 001
15	528 ± 218	1396 ± 395	5 4	11 3	0 0003
30	235 ± 72	435 ± 133	2 3	3.5	0 01
60	133 ± 34	205 ± 73	1 3	1 7	0 08
90	113 ± 27	164 ± 41	1 1	1 3	0.06
120	98 ± 21	138 ± 42	1 1	1 1	0 88
Lactate					
Preinfusion	1086 ± 404	1169 ± 357	1	1	
5	3528 ± 745	3831 ± 1258	3.6	3 3	0.68
15	2757 ± 467	4203 ± 1061	2 7	3 8	0 005
30	1850 ± 353	3192 ± 1137	1 8	2 8	0.001
60	1223 ± 317	2030 ± 689	1 2	1 8	0 0006
90	1021 ± 237	1548 ± 493	1.0	1 4	0.005
120	881 ± 211	1290 ± 485	1 0	1.1	0 36
Lactate-pyruvate ratio			P^b		
Preinfusion	10 1 ± 4 5	9.2 ± 1 8	0 83		
5	2 1 ± 1 0	0 8 ± 0.3	0 0001		
15	5 8 ± 1 9	3 1 ± 0 7	0 0001		
30	8 3 ± 1 8	7 4 ± 1.8	0 22		
60	9 4 ± 1 8	10 1 ± 2.2	0.32		
90	9 2 ± 1.3	9.4 ± 2.1	0 88		
120	9 0 ± 1 3	9 2 ± 1.7	0 79		

[a] *P*-value according to Student's *t*-test, applied to the logarithm of (the serum concentration relative to the preinfusion concentration)
[b] *P*-value according to Student's *t*-test, applied to the logarithm of lactate-pyruvate ratio.

Table 4. Results of linear discriminant analysis control group (n=23) versus Leigh syndrome group (n=9)

Variables used in the procedure	D	Number of misclassifications by resubstituting procedure according to standard linear discriminant function analysis	
		Control group	Leigh group
LL_{00}, LL_{05}, , LL_{90}	15 54	0	0
LL_{05}, LL_{15}	12 13	0	0
LP_{00}, LP_{05}, , LP_{90}	10 67	0	1
LP_{05}, LP_{15}	8 88	2	1

LL = logarithm of lactate level at specific time (00, 05, , 90 min), LP = logarithm of pyruvate level at specific time (00, 05, , 90 min), D = Malanobis distance

$D^2 = \frac{n_1 + n_2}{n_1\ n_2}\ T^2$, in which T^2 = Hotelling test statistic, n_1 = No of controls, n_2 = No of Leigh patients

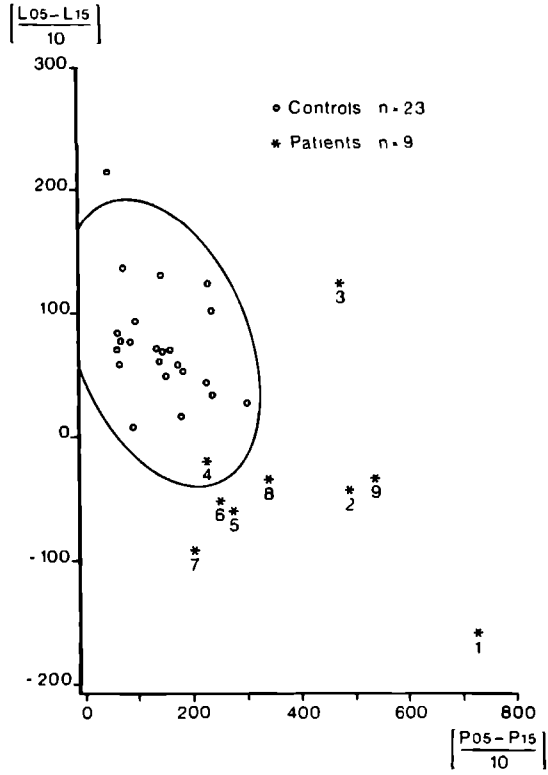

Fig 1. Tolerance region (90%–90%) for the control group based on response parameters $\frac{P_{05} - P_{15}}{10}$ and $\frac{L_{05} - L_{15}}{10}$ $P_{05(15)}$ = pyruvate level 5 (15) min after infusion, $L_{05(15)}$ = lactacte level 5 (15) min after infusion

where we expect the response of the individual control patient will belong to with a certain probability. As statistics for the visualization of such a region we chose the difference in serum levels at t_5 and t_{15} from both lactate and pyruvate response. The ellipsoidal tolerance region R is constructed (Fig 1), according to the method based on the multivariate normal distribution (Chew, 1966), in such a manner that the probability is 0.90 that R contains at least 90% of the individuals in the population of control patients from which our sample is supposed to be drawn at random.

BIOCHEMICAL STUDIES IN TISSUES

The results of biochemical studies have been summarized in Table 5. Pyruvate oxidation by cultured fibroblasts from all 7 patients measured was normal. Liver tissue of all patients measured showed a normal pyruvate carboxylase activity, while the rate of pyruvate oxidation was decreased. Oxidation of pyruvate and malate, and ATP production in muscle tissue had a decreased rate in 7 patients. In cases 1, 4 and 9, this could be explained by abnormalities in the respiratory chain.

DISCUSSION

An intravenous pyruvate loading test was performed in 9 patients with Leigh syndrome (3 confirmed, 6 clinical diagnosis). Preinfusion levels were the same for both patient and control groups. Leigh patients responded quite different to pyruvate loading compared to the control group. Patient response was very similar to the response of Friedreich patients as described by Dijkstra et al (1984).

Pyruvate response was in all patients of the same shape as in controls, but pyruvate levels in the period from 5 to 30 min after administration were significantly elevated in patients when compared to controls (Table 3).

The lactate response curves differ in shape with the control curves in that, in all patients but one, the postinfusion level at t_{15} is higher than the level at t_5. This patient (patient 3), however, shows the highest lactate values of all patients at all sampling points.

With the use of the values at t_5 and t_{15} it proved possible to construct a tolerance region for the control group (Fig 1), which proved its use for diagnostic purposes. As can be seen in this figure, one patient falls in the tolerance region for the controls, but this patient can also be

Table 5. Results of biochemical studies in tissues

	Fibroblasts	Liver	Muscle			
Patient No.	Pyruvate oxidation	Pyruvate carboxylase	Pyruvate oxidation	Pyruvate, malate oxidation	ATP production from pyruvate	Respiratory chain defect
1	N	N	↓	↓	↓	NADH dehydrogenase deficiency
2	–	N	↓	↓	–	NADH dehydrogenase deficiency
3	N	N	–	N	N	N
4	N	N	–	↓	↓	NADH dehydrogenase deficiency
5	N	–	↓	↓	↓	N
6	N	N	–	N	N	N
7	N	N	↓	↓	↓	no localized defect
8	N	N	↓	↓	↓	no localized defect
9	–	–	–	↓	↓	cytochrome oxidase deficiency

N = normal; ↓ = decreased; – = not measured.

discriminated from the controls in that his lactate level rises after t_5 to reach a maximum at t_{15}.

The intravenous pyruvate loading test has in our patients a greater sensitivity than the oral glucose loading test, that yielded abnormal results in only 2 cases, and 24-h lactate excretion in urine that was permanently elevated in 2 patients and showed an intermittent rise in 1 other patient. In nearly all our patients, serum levels of pyruvate and lactate reflect the clinical activity of the disease process (Table 2). In a phase of clinical deterioration, pyruvate and lactate levels show borderline or clear elevations and in the interval between bouts the levels of pyruvate and lactate are normal. So it is important not to rely on one measurement. But even when measured in a phase of clinical deterioration, serum levels of pyruvate and lactate in our patients only seldom give a straight-forward indication of a disturbance of pyruvate metabolism. This is contrary to our findings in CSF, where 6 patients showed clear elevations of pyruvate and lactate levels, while in 2 patients the elevations were borderline and only one patient (patient 8) showed normal lactate and pyruvate values. This illustrates the independence of the CSF lactate level of the serum lactate level, the former reflecting directly brain pyruvate metabolism (Posner and Plum, 1967).

In our opinion, the intravenous pyruvate loading test is a specific and sensitive procedure to detect disturbances of pyruvate metabolism in patients clinically suspected of so-called mitochondrial myopathies (Sengers et al, 1984) and mitochondrial encephalomyopathies (Shapira et al, 1977). In case of a strong clinical suspicion of a disturbance of pyruvate metabolism while routine investigations are not conclusive, as might be the case in quiet phases of the disease, the intravenous pyruvate loading test can have diagnostic value. If routine investigations indicate a disturbance of pyruvate metabolism, intravenous pyruvate loading test will give no additional information.

We have no experience with pyruvate loading in patients with a pyruvate carboxylase deficiency. A normal loading test does therefore not exclude a pyruvate carboxylase deficiency.

If pyruvate and lactate levels of serum and CSF, and 24-h urinary lactate excretion are normal, and oral GTT and intravenous pyruvate loading yield normal results, pyruvate oxidation must be considered undisturbed in muscle and brain tissue.

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CHAPTER VI

MITOCHONDRIAL ENCEPHALOMYOPATHY

Association with an NADH Dehydrogenase Deficiency

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ABSTRACT

A 17-year-old patient had a progressive hypokinetic-rigid syndrome and several other signs and symptoms that indicated central nervous system involvement. Biochemical studies revealed a reduced form of nicotinamide-adenine dinucleotide dehydrogenase deficiency in skeletal muscle. Clinical signs and symptoms, and their association with an established defect of energy metabolism, led us to classify this disorder as a mitochondrial encephalomyopathy of Leigh's type.

INTRODUCTION

Since the first description by Luft et al[1] of mitochondrial dysfunction in a patient with hypermetabolism and muscle weakness, steadily increasing numbers of morphologic, functional, and biochemical abnormalities of skeletal muscle mitochondria have been reported.[2] Price[3] termed this group of disorders 'mitochondrial myopathies'. Analogous to this concept, Shapira et al[4] described the mitochondrial encephalomyopathies, a group of disorders with central nervous system (CNS) and muscle involvement, associated with abnormalities in mitochondrial metabolism and/or structure. Representatives of this group are the syndrome of Alpers,[5] dysmyelination,[2] the syndromes of Leigh[6–9] and Kearns-Shy,[10] myoclonus epilepsy associated with 'ragged-red fibers',[11] and the so-called MELAS (*m*itochondrial myopathy, *e*ncephalopathy, *l*actic *a*cidosis, and *s*trokelike episodes) syndrome.[12] Our patient had a clinical diagnosis of Leigh's syndrome and the reduced form nicotinamide-adenine dinucleotide (NADH) dehydrogenase deficiency in muscle biopsy material.

REPORT OF A CASE

The patient is a 17-year-old boy, the only son of nonconsanguineous parents. The medical history of both parents and their families are unremarkable

Psychomotor development was normal until age 14 years, when a slowly progressive disturbance of motor function, exercise intolerance, and muscular weakness appeared. His performance in school showed a considerable decline, and changes in his conduct, with social withdrawal and aggressive behavior, also developed

Examination revealed a small, thin 17-year-old boy with a poor facial expression and a parkinsonlike posture with bending forward and semiflexion of arms and legs. His height was 163 cm (2 5th percentile); his weight was 41 kg (2.5th percentile), and his skull circumference was 52.5 cm (tenth percentile) There was no intellectual impairment, full-scale IQ (Wechsler Intelligence Scale for Children-Revised and Wechsler Adult Intelligence Scale) was 115. Irregular breathing with frequent sighing occurred, as did bouts of sinus tachycardia of 140 beats per minute and sinus bradycardia of 50 beats per minute

Speech showed a marked dysarthria Automatic movements were diminished, intentional, deliberate movements were slow and clumsy Investigation of muscle tone revealed a generalized rigidity with a cogwheel phenomenon No resting tremor was apparent, but the hands showed athetotic movements Gait was broad based with marked starting difficulty, and steps were small Eye movements were jerky with loss of smooth pursuit. Cerebellar involvement was indicated by ataxia, intention tremor, and dysdiadochokinesis There was generalized muscular weakness Tendon reflexes were hyperactive with bilateral Babinski's signs The Oseretsky motor development and performance test showed his motor development level to be that of a 7-year-old.

ELECTROPHYSIOLOGIC AND RADIOLOGIC INVESTIGATIONS

Electroencephalogram, electromyogram and results from nerve conduction

velocity tests were normal. Somatosensory, brainstem auditory and visual evoked potentials fell within the normal ranges. Roentgenograms of skull, chest, hand, and vertebral column, and cerebral computed tomographic scans showed no abnormalities. Electrocardiogram was normal and the echocardiogram showed no signs of cardiomyopathy.

ROUTINE AND SPECIFIC LABORATORY INVESTIGATIONS

Results from standard blood and urinary studies, including hematologic evaluation, renal and hepatic functions, serum protein levels, serum protein electrophoresis, and immunoelectrophoresis were normal. Values were also normal for the following: blood pH; carbon dioxide pressure and bicarbonate; serum lipids; short-chain fatty acids and carnitine (total and free); serum uric acid and ammonia; organic acids and amino acids in plasma and urine; lysosomal enzymes in leukocytes; creatine kinase; serum copper, zinc, manganese, and ceruloplasmin; urinary copper excretion; thyroid and adrenal functions; and blood levels of growth hormone, thiamine, pyridoxine, and cobalamin. Appropriate studies ruled out immunologic and chronic infectious diseases and disorders caused by toxic agents. In cerebrospinal fluid (CSF), cell number, protein content, protein electrophoresis and immunoelectrophoresis findings, and values for homovanillic acid and 5-hydroxyindolacetic acid, and minerals were normal.

ENERGY METABOLISM

Serum levels of glucose, pyruvate, lactate, β-hydroxybutyrate, and acetoacetate were normal under resting conditions. The lactate-pyruvate and β-hydroxybutyrate-acetoacetate ratios were normal. Urinary lactate excretion was normal. Oral glucose tolerance test (1.75 g/kg) revealed a normal glucose and pyruvate response, but lactate level increased from 1,16 to 2.3 mmol/L (1.16 to 2.3 mEq/L) (normal, up to 1.7 mmol/L [1.7 mEq/L]). The McArdle-Fischbein ischemic exercise test[13] yielded a normal rise of serum lactate and ammonia levels. Moderate physical exercise, eg, walking for a few minutes, gave a marked increase in serum lactate level to 4.74 mmol/L (4.74 mEq/L), in the serum pyruvate level to 0.22 mmol/L (0.22 mEq/L) (normal, 0.06 to 0.16 mmol/L [0.06 to 0.16 mEq/L]), and in the lactate-pyruvate ratio to 21.5 (normal, up to 15), and a metabolic acidosis with a pH of 7.23 and a base excess of -12.4. After intravenous pyruvate loading (500 mg/kg),[14] there was a normal pyruvate response, but lactate response was abnormal (Fig 1).

The CSF glucose, acetoacetate and β-hydroxybutyrate levels were normal

under resting conditions and after moderate physical exercise. The pyruvate level (0.15 mmol/L [0.15 mEq/L]; normal, 0.09 to 0.13 mmol/L [0.09 to 0.13 mEq/L]) and the lactate level (3.21 mmol/L [3.21 mEq/L]; normal, 1.2 to 1.6 mmol/L [1.2 to 1.6 mEq/L]) were elevated, with an increase of the lactate-pyruvate ratio (20.8; normal, up to 15).

HISTOPATHOLOGIC STUDIES

Histochemical studies of quadriceps muscle, obtained at biopsy, showed no abnormalities. No ragged-red fibers were noted in the trichromic-stained section; no Sudan black-positive droplets were seen. Ultrastructural studies revealed no mitochondrial abnormalities.

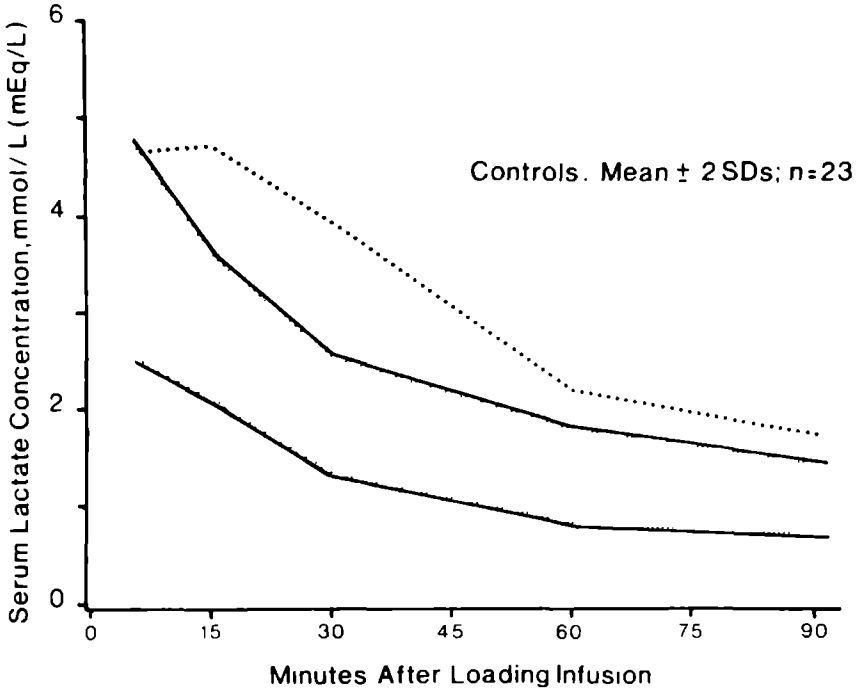

Fig 1. *Lactate response to pyruvate loading (500 mg/kg). Preinfusion level 1.6 mmol/L (1.6 mEq/L).*

BIOCHEMICAL STUDIES

METHODS

Enzymatic studies were performed in cultured fibroblasts and in liver and muscle that were obtained at biopsy. Oxidative metabolism was evaluated in fibroblasts by measuring the radioactive carbon dioxide ($^{14}CO_2$) production rate from radioactive carbon 14 in the No. 1 position-labeled pyruvate ([1-^{14}C]pyruvate) and [2-^{14}C]pyruvate,[15] and the activities of cytochrome oxidase and citrate synthase. Pyruvate carboxylase activity was measured in liver homogenate,[16] using a regenerating system for acetylcoenzyme A.[17] Pyruvate oxidation rate, and activities of citric acid cycle and respiratory chain were evaluated by measuring $^{14}CO_2$ production from [1-^{14}C]pyruvate and malate uniformly labeled with ^{14}C (malate ^{14}C [ul]) in fresh muscle homogenate.[18] The capacity of muscle to generate adenosine triphosphate (ATP) and creatine phosphate (CrP) was investigated under conditions in which adenylate kinase was inhibited.[19] In muscle homogenate, frozen at -70°C, carnitine content[20] and activities of citrate synthase,[21] cytochrome *c* oxidase,[22] succinate:cytochrome *c* oxidoreductase,[23] NADH:O_2 oxidoreductase,[24] and NADH:Q_1 oxidoreductase[24] were measured. Cytochrome content[25] was measured in isolated muscle mitochondria in the presence of succinate and cyanide, and subsequently in the presence of antimycin A (1 μg). Protein was assayed according to Lowry et al.[26]

RESULTS

In fibroblasts, oxidation rates were normal: [1-^{14}C]pyruvate: 59 nmol/h.mg of protein (control range, 25 to 96 nmol/h.mg of protein; n=23); [2-^{14}C]pyruvate: 23 nmol/h.mg of protein (control range, 12 to 35 nmol/h.mg of protein; n=13). Activities of cytochrome oxidase (22 mU/mg of protein [control range, 11 to 43 mU/mg of protein; n=15]) and citrate synthase (41 mU/mg of protein [control range, 26 to 63 mU/mg of protein; n=15]) were also within the normal ranges. In liver tissue, pyruvate carboxylase activity was normal: 19 mU/mg of protein (control range, 13 to 40 mU/mg of protein; n=15). The $^{14}CO_2$ production rates from [1-^{14}C]pyruvate and malate ^{14}C (ul) by muscle homogenate showed a marked decrease under all experimental conditions (Table 1). The ATP + CrP production with pyruvate as substrate was also strongly decreased. The ratio of the ATP + CrP production to the arsenite-sensitive pyruvate oxidation[27] was slightly decreased. Total carnitine content (carnitine and acylcarnitines) in muscle homogenate was normal: 3.1 μmol/g wet weight

Table 1. Results of biochemical studies in muscle tissue

Study*	Patient	Controls Range	Mean ± SD	No.
Oxidation (600 g supernatant)				
[1-^{14}C]pyruvate + malate	69†	273-705	473 ± 117	20
[1-^{14}C]pyruvate + carnitine	103†	266-941	504 ± 169	20
Malate ^{14}C (ul) + pyruvate + malonate	69†	320-996	621 ± 211	20
Malate ^{14}C (ul) + acetylcarnitine + malonate	69†	317-1155	575 ± 220	20
Malate ^{14}C (ul) + acetylcarnitine + arsenite	34†	198-274	294 ± 80	19
ATP Metabolism (600 g supernatant)				
ATP + CrP production from pyruvate	496‡	3354-9993	5910 ± 2168	20
ATP + CrP production / pyruvate oxidation	7 2	8.8-15 0	11 8 ± 1 8	20
Enzyme activities (600 g supernatant)				
Citrate synthase	57§	48-146	77 ± 33	18
Cytochrome *c* oxidase	166§	73-284	194 ± 92	39
Succinate cytochrome *c* oxidoreductase	22‖	22-84	50 ± 18	18
NADH O_2 oxidoreductase	8 5¶	14-45	29 ± 10	12
NADH.Q_1 oxidoreductase	5 0¶	8 7-25	18 ± 5	11
Cytochromes (isolated mitochondria)				
Cytochrome aa_3	486#	271-585	430 ± 100	14
Cytochrome *b*	488#	317-511	400 ± 90	14
Cytochrome $c + c_1$	531#	365-923	609 ± 166	14
Cytochrome *c* oxidase	4 26**	1 29-3 43	2.34 ± 0 54	16

* [1-^{14}C] indicates radioactive carbon 14 in the No 1 position-labeled, ^{14}C (ul), uniformly labeled with ^{14}C, ATP, adenosine, triphosphate, CrP, creatine phosphate, NADH, the reduced form of nicotinamide-adenine dinucleotide, Q_1, ubiquinone-1 (analogon of coenzyme Q).
† Nanomoles of $^{14}CO_2$ per hour per milligram of protein.
‡ Nanomoles per hour per milligram of protein.
§ Milliunits per milligram of protein.
‖ Nanomoles of cytochrome *c* reduced per minute per milligram of protein.
¶ Nanomoles of NADH oxidized per minute per milligram of protein.
# Picomoles per milligram of protein
** Units per milligram of protein

(control range, 2.7 to 4.6 μmol/g wet weight; n=21). The specific activities of the mitochondrial enzymes in muscle homogenate are also shown in Table 1. Citrate synthase, used as a mitochondrial reference enzyme, had a normal activity in muscle homogenate. Concerning the respiratory chain, normal activities for cytochrome *c* oxidase and succinate:cytochrome *c* oxidoreductase and normal concentrations of the cytochromes were found. The activities of both NADH:O_2 and NADH:Q_1 oxidoreductase, however, were decreased to 30% of mean control activity (Table 1).

We present a 17-year-old patient with a progressive hypokinetic-rigid syndrome, athetosis of the hands, pyramidal and cerebellar signs, periodic hyperventilation, abnormalities of cardiac rhythm, and exercise intolerance.

The abnormally high increase in the blood lactate level after oral glucose loading, in the lactate and pyruvate levels after moderate physical exercise, and in the lactate level after intravenous pyruvate loading are indications of a disturbance of pyruvate metabolism. The clearly decreased oxidation of pyruvate in muscle tissue confirms this (Table 1). A deficiency of the pyruvate dehydrogenase complex can be excluded, because in that case the oxidation of malate in the presence of acetylcarnitine as cosubstrate should be undisturbed. The oxidation rates of pyruvate and malate, however, were lowered under all conditions tested. This finding points to a defect in a pathway necessary for the oxidation of pyruvate and malate under all experimental conditions, eg, the respiratory chain. Indeed, the activity of NADH:O_2 oxidoreductase, reflecting the overall capacity of the respiratory chain (Fig 2),[24] is too low (Table 1). The defect can be pinpointed to the NADH dehydrogenase (complex I) activity, as NADH:Q_1 oxidoreductase has a decreased activity, while all other components of the respiratory chain show normal activities and/or concentrations (Fig 2). The increase in CSF lactate and pyruvate levels makes it likely that the same defect is present in the CNS.

In fibroblasts, no abnormalities in the mitochondrial oxidative metabolism could be demonstrated, which has its negative consequence for antenatal diagnosis.

In the literature, several patients with complex I deficiencies have had their cases reported.[9,28–36] In one of these patients,[9] a deficiency of NADH dehydrogenase has been established recently by direct assay.[24] To our knowledge, there is direct proof of an NADH dehydrogenase deficiency in only one other patient.[35] Clinically, there is a myopathy without neurologic signs and symptoms in six patients, and in the other four patients,[9,32,33,36] signs and symptoms originate predominantly from the CNS.

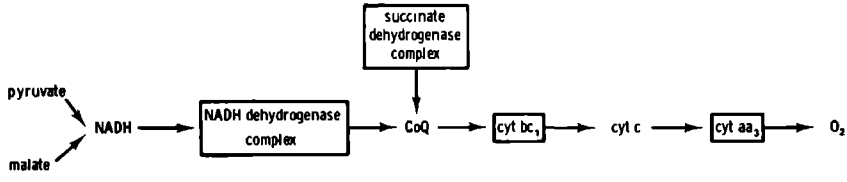

Fig 2. Scheme of respiratory chain. NADH indicates reduced form of nicotinamide-adenine dinucleotide, CoQ, coenzyme Q; cyt bc_1, cytochrome bc_1; cyt c, cytochrome c; cyt aa_3, cytochrome aa_3

Table 2. Summary of clinical signs and symptoms of our patient compared with results of a literature study of 24 proven juvenile Leigh patients

	Patient	Literature
Respiratory signs and symptoms	+	54%
Cardiac signs and symptoms	+	25%
Ocular signs	+	79%
Pyramidal signs	+	88%
Extrapyramidal signs	+	46%
Cerebellar signs	+	54%
Exercise intolerance	+	50%
Decompensation on infection	+	42%

Of the latter group, one is suffering from Alpers' disease,[32] and in two patients the diagnosis of Leigh's syndrome is proved.[9,36]

In our patient and in some of the patients whose cases are reported in literature with a complex I deficiency,[9,30,32,36] there are no gross structural mitochondrial abnormalities, while the other patients all show a wide range of morphologic mitochondrial abnormalities. This illustrates that there is no specific correlation between defects of mitochondrial metabolism and alteration in mitochondrial structure.[37]

In our patient, a clinical and biochemical myopathy and CNS involvement are apparent. We consider him suffering from mitochondrial encephalomyopathy,[4] compatible with Leigh's syndrome. Signs and symptoms combined with a proved defect of energy metabolism led us to this conclusion. In Table 2, signs and symptoms of our patient are compared with signs and symptoms of 24 patients with proved juvenile Leigh's syndrome (age at onset between 4 and 16 years) whose cases were derived from the literature. As can be seen in Table 2, extrapyramidal signs are present in half of the patients with juvenile Leigh's syndrome.

Leigh's syndrome has been associated with deficiencies of pyruvate carboxylase,[6] pyruvate dehydrogenase complex,[7] cytochrome *c* oxidase,[8] NADH dehydrogenase,[9] and a disturbance in the activation of the pyruvate dehydrogenase complex.[38]

Until now, the cases of more than 30 patients with respiratory chain deficiencies have been reported.[39] Recognition of clinical entities in electron transport chain defects is difficult, due to the great variability in clinical presentation of a defect and the relatively small number of patients whose cases have been reported until now. At present, classification can best be made on biochemical grounds.

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H. Alfons D. Walder, MD, PhD, performed the muscle biopsy, Henk J. ter Laak, PhD, performed the histochemical studies, and Ad M. Stadhouders, PhD, performed the ultrastructural studies.

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CHAPTER VII

SUBACUTE NECROTIZING ENCEPHALOMYELOPATHY (LEIGH SYNDROME) ASSOCIATED WITH DISTURBED OXIDATION OF PYRUVATE, MALATE AND 2-OXOGLUTARATE IN MUSCLE AND LIVER

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ABSTRACT

We studied a 17-year-old girl with subacute necrotizing encephalomyelopathy (Leigh syndrome). Lactate and pyruvate levels were increased in serum and cerebrospinal fluid. The oxidation rates of all substrates tested, i.e. pyruvate in liver, and pyruvate, malate and 2-oxoglutarate in muscle, were decreased, as was the production of adenosine triphosphate plus creatine phosphate. Cytochrome content was normal. The data imply a defect in oxidative phosphorylation, outside the cytochrome region.

INTRODUCTION

In 1951, Leigh (1) described a 7-month-old boy with a unique central nervous system pathology, which he termed subacute necrotizing encephalomyelopathy (SNE, Leigh syndrome). The diagnosis still is neuropathologic, characterized by spongy dystrophy of the neuropil, pronounced proliferation of endothelial cells in the walls of small blood vessels and relative sparing of neuronal cells. The lesions show a bilateral, often symmetrical, distribution and are found most characteristically in the tegmentum of the brainstem and walls of the third ventricle (2).

Since the description of a pyruvate carboxylase deficiency in a patient with Leigh syndrome by Hommes et al (3) in 1968, several investigators have reported disorders of pyruvate metabolism or of the respiratory chain. Deficiencies of pyruvate carboxylase (3-8), the pyruvate dehydrogenase complex (9-17) and cytochrome *c* oxidase (18,19) have been described. Cooper et al (20) demonstrated a factor in body fluids that inhibits thiamine diphosphate-ATP phosphoryltransferase; it blocks *in vitro* synthesis of thiamine triphosphate, thus implicating abnormal thiamine metabolism as a pathogenic factor (21).

We now describe a 17-year-old girl with the classic pathology of SNE, and an abnormal oxidative phosphorylation.

CASE REPORT

This patient was the youngest daughter of healthy, nonconsanguineous parents There were 3 healthy siblings Her youngest brother lost vision for 5 years, with bilateral optical atrophy of unknown origin In this case, neurological examination and routine chemical investigations revealed no abnormalities Lactate and pyruvate in serum and cerebrospinal fluid (CSF) were not measured At age 9, the boy was nearly blind and died in the course of an intercurrent infection Cerebral autopsy was not performed.

Our patient showed normal development and had an uneventful medical history until age 4, when she was seen by a neurologist because she had had difficulty in walking for a few months Mild spasticity of both legs, hyperactive tendon reflexes of the legs, ankle clonus, and Babinski signs were seen Apart from slight bilateral horizontal nystagmus, clumsy gait was the only sign for the next 13 years There were no exacerbations on intercurrent infections No exercise intolerance was noted.

At age 17, the gait disorder gradually became more pronounced, and the right arm and hand were paretic. There were no visual symptoms On examination, there was no intellectual impairment There was external ophthalmoplegia, with nystagmus of the left eye in all directions. Fundoscopy was normal. The right arm showed a mild paresis The paresis of the legs had increased. Tendon reflexes of the arms were also hyperactive

Three months after onset of the exacerbation, she had difficulty in breathing and became comatose On artificial respiration, consciousness cleared in so far as she could carry out simple tasks, such as moving the limbs, but spontaneous breathing did not recover After a few days, coma gradually deepened, tendon reflexes were lost, but plantar responses remained extensor. Pupillary reflexes and brainstem reflexes could not be elicited After 16 weeks of artificial respiration, all treatment was stopped and the patient died.

ELECTROPHYSIOLOGICAL AND RADIOLOGICAL INVESTIGATIONS

A few days after the onset of coma, EEG showed diffuse slowing (1-3 Hz) with no signs of focal pathology. EMG and nerve conduction velocities were normal. The blink reflex was normal during the first weeks, but gradually disappeared. No evoked potentials were recorded. Cerebral CT and 4-vessel angiography showed no abnormalities.

ROUTINE AND SPECIFIC LABORATORY INVESTIGATIONS

Results from standard blood and urinary studies, including hematologic evaluation, renal and hepatic functions, serum protein, and serum protein electrophoresis, were normal. Other normal studies included serum pH, pCO_2 and bicarbonate, serum glucose, serum lipids and short-chain fatty acids, organic acids and amino acids in plasma and urine, lysosomal enzymes, creatine kinase, serum copper and ceruloplasmin. Serum levels of lactate and pyruvate were measured several times and were normal or moderately elevated (Table 1); 24-hr urinary lactate excretion was normal. Appropriate studies ruled out endocrinological, immunological and chronic infectious diseases, deficiencies, and disorders caused by toxic agents.

CSF cell count, protein content, protein electrophoresis and immunoelectrophoresis, minerals, glucose, acetoacetate, and β-hydroxybutyrate were normal. Lactate and pyruvate levels were elevated, with an increased lactate-pyruvate ratio (Table 1).

NEUROPATHOLOGIC EXAMINATION

Macroscopic examination of brain and spinal cord revealed a spongy aspect of the walls of the third ventricle (Fig 1), the tegmental region of

Table 1. Lactate and pyruvate levels in serum and cerebrospinal fluid (CSF) of patient and controls

		Lactate[a]	Pyruvate[a]	Ratio Lactate-Pyruvate
Serum	Patient[b]	950-2330	106-200	8.0-15.0
	Controls (n=200)	600-1200	75-120	up to 15
CSF	Patient[b]	2520-4470	143-198	17.6-22.6
	Controls (n=200)	1200-1600	85-132	up to 15

[a] μmol/l; [b] range, based on measurements in 4 samples.

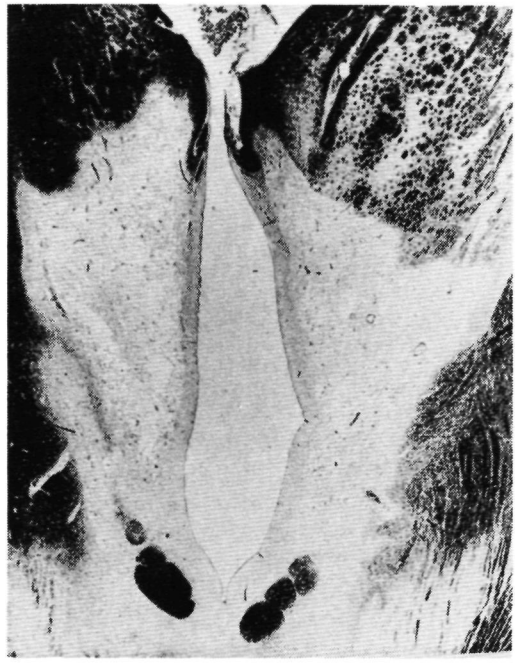

Fig 1. Spongy degeneration of the walls of the third ventricle. Klüver-Barrera.

mesencephalon, pons and medulla oblongata, and the central part of the spinal cord. On light microscopic examination, the hypothalamic area, walls of the third ventricle, tegmental region of mesencephalon and pons, olivary nuclei, nucleus dentatus, medulla oblongata, and central part of the spinal cord showed pathological changes, characterized by microvacuolization and cyst formation, proliferation of protoplasmic and fibrocytic astrocytes, and vascular and endothelial proliferation. Within the lesions, which had a punched-out aspect, the ganglion cells remained relatively intact. There were no hemorrhagic changes and no inflammatory signs. Histochemical and ultrastructural studies of the frontal cortex revealed no pathological changes. No mitochondrial abnormalities were seen.

BIOPSY STUDIES

All biopsy specimens were taken while the patient was in coma.

MORPHOLOGICAL STUDIES

Microscopic and histochemical studies were performed on liver tissue. Histochemical and ultrastructural studies were performed on muscle tissue.

BIOCHEMICAL STUDIES

Material

Biochemical studies were performed in liver and muscle homogenates, and in isolated muscle mitochondria. Two specimens of quadriceps muscle were available for biochemical studies, obtained one and two months, respectively, after onset of coma.

Methods

Pyruvate metabolism in liver tissue. Pyruvate oxidation rate in a fresh liver tissue homogenate was studied by measuring $^{14}CO_2$ production from [1-^{14}C]pyruvate (18). Pyruvate carboxylase (E.C.6.4.1.1) activity was measured in the same liver homogenate (22) after storage at -70°C, using a regenerating system for acetyl-CoA (23).

Mitochondrial oxidative metabolism in muscle tissue. Pyruvate oxidation rate and activities of citric acid cycle and respiratory chain in fresh muscle homogenate were evaluated by measuring $^{14}CO_2$ from [1-^{14}C]pyruvate, [1-^{14}C]2-oxoglutarate and [U-^{14}C]malate (24). The capacity of muscle to synthesize adenosine triphosphate (ATP) and creatine phosphate was investigated under conditions in which the adenylate kinase activity was inhibited (25). In frozen muscle homogenate, carnitine content (26) and activities of carnitine palmitoyltransferase (E.C.2.3.1.21) (exchange reaction at 0.8 mM palmitoylcarnitine) (27), citrate synthase (E.C.4.1.3.7) (28) and cytochrome *c* oxidase (E.C.1.9.3.1) (29) were measured. The assay medium for succinate-cytochrome *c* oxidoreductase activity contained 0.05 mM cytochrome *c*, 2 mM KCN and 2 μg/ml rotenone. Extinction was followed at 550 nm; extinction coefficient used was 21.1 $mM^{-1}.cm^{-1}$. The assay medium for succinate dehydrogenase (E.C.1.3.99.1) activity contained 0.05 mM dichlorophenolindophenol. Extinction was followed at 600 nm; extinction coefficient used was 19.1 $mM^{-1}.cm^{-1}$. For measurement of succinate-cytochrome *c* oxidoreductase and succinate dehydrogenase activity, muscle homogenates were preincubated with 20 mM succinate in 50 mM KP_i, pH 7.4. The dehydrogenase activity was corrected for the activity of samples preincubated with malonate instead of succinate. Both assays were performed at 25°C. In isolated muscle mitochondria (24), cytochrome *c* oxidoreductase activity (29), cytochromes (30), and oxygen consumption

at 37°C with 1 mM pyruvate and 1 mM malate as substrates (31), were measured.

Protein determination. Protein was assayed according to Lowry et al (32).

RESULTS

MORPHOLOGICAL STUDIES

Microscopic and histochemical studies of liver revealed no abnormalities. Histochemical and ultrastructural studies of quadriceps muscle showed no abnormalities, particularly no 'ragged-red fibers' were seen in the trichromic stained section, and electron microscopy revealed no abnormal mitochondria.

BIOCHEMICAL STUDIES

Pyruvate carboxylase activity in liver homogenate was normal (37.0 nmol/min/mg protein; controls 28.2 ± 12.1, mean ± SD; n=16), and $^{14}CO_2$ production rate from [1-^{14}C]pyruvate + malate was decreased (Table 2). $^{14}CO_2$ production rate from [1-^{14}C]pyruvate, [1-^{14}C]2-oxoglutarate and [U-^{14}C]malate in muscle homogenate was markedly decreased (Table 2). ATP production with pyruvate, malate or 2-oxoglutarate as substrates was lower than in control muscle homogenates (Table 3). The ratio of the amount of ATP produced to the amount of pyruvate oxidized was not decreased (33). Total carnitine content (carnitine + acylcarnitines) in muscle homogenate was normal. The specific activities of the mitochondrial enzymes in muscle homogenate are shown in Table 4. Cytochrome *c* oxidase activity

Table 2. $^{14}CO_2$ production from various ^{14}C-labeled substrates in liver and muscle tissue homogenates of patient and controls

	Substrate	Patient		Controls
Liver	[1-^{14}C]pyruvate + malate	13 6[a]		45-205 (n=13)
		Biopsy 1	Biopsy 2	
Muscle	[1-^{14}C]pyruvate + malate	54[a]	73[a]	243-729 (n=41)
	[1-^{14}C]pyruvate + carnitine	98	143	221-838 (n=39)
	[1-^{14}C]pyruvate + malate + malonate	31		214-687 (n=36)
	[U-^{14}C]malate + pyruvate + malonate	90	136	390-1172 (n=39)
	[U-^{14}C]malate + acetylcarnitine + malonate	108		352-1155 (n=39)
	[U-^{14}C]malate + acetylcarnitine + arsenite	81		172-492 (n=36)
	[1-^{14}C]2-oxoglutarate	134		341-1229 (n=15)

[a] nmol/hr/mg protein.

Table 3. Adenosine triphosphate (ATP) plus creatine phosphate production after incubation of muscle homogenates of patient and controls with various substrates in the presence or absence of specific inhibitors

Substrate	Inhibitor	Patient	Controls
Pyruvate + malate	-	42[a]	78-242 (n=13)
Acetylcarnitine + malate	Arsenite	21	36-69 (n=11)
2-Oxoglutarate	Malonate	10	35-79 (n=6)

[a] nmol/min/mg protein.

Table 4. Activities of mitochondrial enzymes in muscle homogenates of patient and controls

	Patient	Controls
Carnitine palmitoyltransferase	0.23[a]	0 33-0 97 (n=14)
Citrate synthase	44	27-77 (n=18)
Cytochrome *c* oxidase	65	73-284 (n=39)
Succinate - cytochrome *c* oxidoreductase	7.3	11-36 (n=14)
Succinate dehydrogenase	7.2	6-16 (n=6)

[a] nmol substrate converted/min/mg protein

in isolated muscle mitochondria was normal (1315 nmol/min/mg protein; controls 1288 - 3427, range; n=19). The contents of cytochrome *b*, *c* + c_1 and aa_3 were normal. Oxygen consumption by isolated muscle mitochondria with pyruvate and malate as substrates, was decreased to about one tenth of normal (2.5 μg-atom O/hr/mg protein; controls 23.0 ± 4.8, mean ± SD; n=4).

DISCUSSION

Leigh syndrome can be transmitted as an autosomal recessive trait (34). It is likely, therefore, that this patient's brother, though presenting with a different clinical picture, also suffered from SNE. Optical atrophy and blindness have been reported in SNE (2). The insidious onset and the chronic unremitting course in our patient, ending with a subacute exacerbation and coma, is seen in some cases of SNE (34,35).

The intermittent rise of serum levels of lactate and pyruvate and, especially, the high CSF lactate and pyruvate levels in our patient pointed to a primary or secondary defect in pyruvate oxidation.

Pyruvate oxidation was decreased in both liver and muscle. In muscle homogenate, $^{14}CO_2$ production from malate and 2-oxoglutarate was decreased too. Furthermore, ATP production with these compounds as substrates was decreased. The efficiency of oxidative phosphorylation (i.e. the amount of ATP produced per molecule substrate oxidized) was not affected, indicating that muscle mitochondria were well coupled. Oxidation

rates remain lowered if expressed on base of mitochondrial marker enzymes. As the mutual ratios of these enzymes (Table 4) are normal, the low normal or decreased activities point to a low mitochondrial content in the homogenate, and not to one or more defective enzymes.

These findings, combined with a very low O_2 consumption and normal cytochrome levels in isolated mitochondria, can be explained by an abnormality in a common metabolic step (24,36). A defect in the NADH-CoQ complex, in the ADP-ATP translocator, or in the F_1-ATPase activity has to be considered (Fig 2). As succinate-cytochrome *c* oxidoreductase and succinate dehydrogenase are not deficient (Table 4), a possible defect in the NADH-CoQ complex is restricted to the pathway in which the reduction equivalents are transported from NADH to CoQ, excluding CoQ itself.

The high CSF lactate and pyruvate levels make it probable that oxidative phosphorylation is abnormal in brain as well as in liver and muscle.

Ours is the first report of a defect in oxidative phosphorylation outside the cytochromes in a patient with Leigh syndrome. Several authors (36-39) have described patients with defects in the first part of the respiratory chain, but the clinical picture in these cases differs from our patient in that a clinical myopathy is almost always present.

Our findings stress again the fact that SNE is a syndrome of different causes, based on a defect in aerobic energy metabolism.

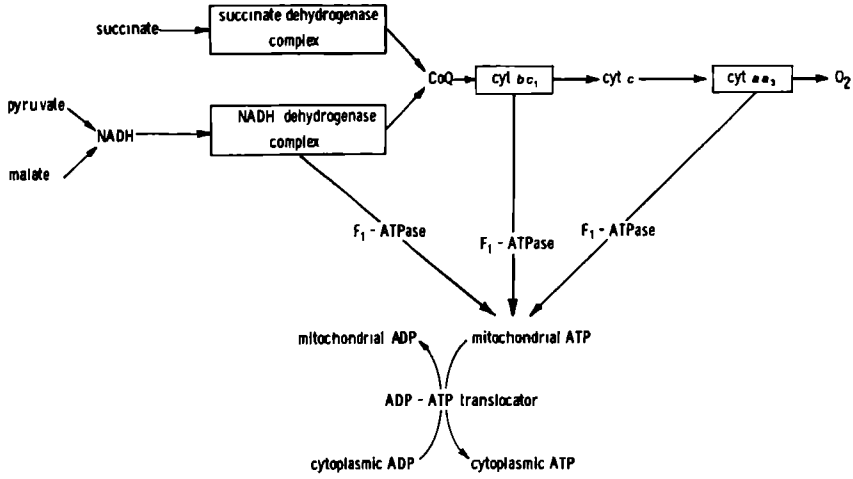

Fig 2. Scheme of the electron flow through the mitochondrial respiratory chain, and the synthesis and transport of adenosine triphosphate (ATP).

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ADDENDUM

DEFECT OF NADH DEHYDROGENASE IN LEIGH SYNDROME

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LETTER TO THE EDITOR

Recently, we reported a patient with Leigh syndrome in this journal (1). Biochemical studies gave indirect evidence of a NADH dehydrogenase deficiency.

In skeletal muscle 600 x g supernatant of this patient, stored at -70°C, we measured activities of NADH:O_2 oxidoreductase and NADH:Q_1 oxidoreductase as described by Fischer et al (2). The NADH:O_2 oxidoreductase activity was determined in the presence of cytochrome *c*. The rotenone-sensitive NADH oxidation by both enzyme systems was determined at 340 nm (2). NADH:O_2 oxidoreductase activity was 2.1 nmol NADH oxidized/min/mg protein (control range 4.5 - 22.9, n=7) and NADH:Q_1 oxidoreductase activity was 3.8 nmol NADH oxidized/min/mg protein (control range 10.6 - 22.1, n=7). NADH:O_2 oxidoreductase activity reflects the oxidizing capacity of the respiratory chain as a whole. The use of Q_1, a water soluble coenzyme Q analogue, as electron acceptor allows a direct estimation of NADH dehydrogenase activity.

The results of our study show that there is a defect in the NADH dehydrogenase, one of the possible causes previously proposed (1). This holds at least for skeletal muscle of the patient, but likely also for brain and liver tissue (1).

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CHAPTER VIII

DISTURBED OXIDATIVE METABOLISM IN SUBACUTE NECROTIZING ENCEPHALOMYELOPATHY (LEIGH SYNDROME)

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ABSTRACT

Several disorders of oxidative metabolism have been described in association with subacute necrotizing encephalomyelopathy (SNE) or Leigh syndrome. We present an eight-year-old girl with a mild spastic paraparesis and clinical deterioration on intercurrent infections. One sib died of SNE proven by autopsy. Biochemical examination of muscle tissue points to a disturbance in the process of oxidative phosphorylation due to a disturbed oxidation of NADH. The biochemical disorders associated with SNE are reviewed. The relation of SNE to the concepts of encephalomyopathy and mitochondriopathy is discussed.

INTRODUCTION

Subacute necrotizing encephalomyelopathy (SNE) or Leigh syndrome is a degenerative disorder of the central nervous system (Leigh, 1951). The diagnosis can only be confirmed by postmortem examination and is based on well-characterized neuropathologic criteria (Pincus, 1972). Clinical signs and symptoms, and course of the disease show a considerable variability (Pincus, 1972).

Different defects of oxidative metabolism have been reported in association with Leigh syndrome (Hommes et al, 1968; Farmer et al, 1973; Willems et al, 1977).

We present an eight-year-old girl, whose brother died of SNE. Investigation of aerobic energy metabolism in muscle tissue pointed to a disturbance in the process of oxidative phosphorylation.

CASE REPORT

Our patient is the youngest daughter of healthy, nonconsanguineous parents She had an uneventful history until she was eighteen months of age At that time she began to walk, but soon it appeared that she had a spastic ataxic walking pattern One year later she was examined at our department. She was a small, nice looking thirty-one-month-old girl, functioning on a normal mental level Weight, height and skull circumference were less than the tenth percentile No dysmorphic features were present Internal examination was normal. Pulse rate under resting conditions varied between 90 and 180/min Mild exercise intolerance was evident. On neurologic examination the following abnormalities were noted· there was spasticity of the legs, and gait was spastic ataxic with bilateral circumduction and dysmetria Tendon reflexes of the legs were hyperactive and the plantar responses were extensor Between ages two and a half and eight years the clinical picture has remained fairly stable, though there have been a few temporary deteriorations on intercurrent infections, that required hospitalization These deteriorations were characterized by aggravation of the neurologic disturbances, apathy, vomiting and Cheyne-Stokes respiration, total recovery usually taking a few months.

Family history. The pedigree is shown in Figure 1 There were two spontaneous abortions at three months amenorrhea The oldest sib is a healthy fifteen-year-old girl The other sister had the same spastic ataxic walking pattern as our patient. At age three years, she got an otitis media with high fever. In one week she deteriorated rapidly, lapsed into coma and died of breathing insufficiency. Brain autopsy was not performed

Patient's brother was noted to have an elevated muscle tone of the legs at age six weeks. This was attributed to a birth trauma caused by difficult delivery due to pelvic contraction. Motor development was slow but within normal range. At age twenty-one months, the boy went through a viral infection with high fever, after which he could not walk without help anymore There proved to be a pronounced spasticity of the legs, with hyperactive tendon reflexes and extensor plantar responses. Biochemical investigations of serum and cerebrospinal fluid (CSF) showed no abnormalities The levels of lactate and pyruvate were not measured. Twenty-four-hour urinary lactate excretion was normal. Radiologic examination of skull and vertebral column, and cerebral CT scanning, EEG and EMG were all normal At age three and a half years, again following an episode of high fever, the boy went into coma, preceded by a few seizures of the grand-mal type. Artificial respiration was necessary due

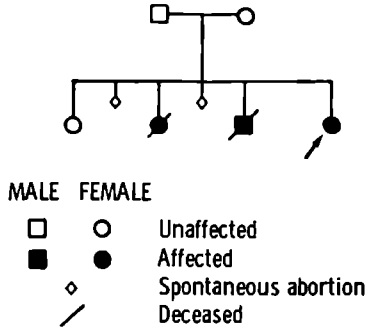

Fig 1. Pedigree of the family.

to respiratory insufficiency. Six weeks later he died. At brain biopsy, an edematous brain was seen, weighing 1010 g, with the classical pathology of SNE.

ELECTROPHYSIOLOGIC AND RADIOLOGIC INVESTIGATIONS

EEG, EMG and nerve conduction velocities were normal. Evoked potentials (somatosensory evoked potentials, brainstem evoked potentials and visual evoked potentials) fell within the normal ranges. Radiograms of skull, chest and vertebral column, and cerebral CT scan showed no abnormalities. ECG and echocardiogram were normal.

ROUTINE AND SPECIFIC LABORATORY INVESTIGATIONS

Results from standard blood and urinary studies, including hematologic evaluation, renal and hepatic functions, serum protein, and serum protein electrophoresis, were normal. Other studies, including serum pH, pCO_2 and bicarbonate, serum glucose, serum lipids and short-chain fatty acids, organic acids and aminoacids in plasma and urine, lysosomal enzymes, creatine kinase, serum copper and ceruloplasmin, showed also normal results. Serum levels of lactate and pyruvate were normal or moderately elevated (Table 1); twenty-four-hour urinary lactate excretion was normal. Appropriate studies ruled out endocrinologic, immunologic and chronic infectious diseases, deficiencies, and disorders caused by toxic agents.

CSF cell count, protein content, protein electrophoresis and immune-electrophoresis, minerals, glucose, acetoacetate, and β-hydroxybutyrate were normal. Lactate and pyruvate levels, and the lactate-pyruvate ratio were normal or moderately elevated (Table 1).

Table 1. Lactate and pyruvate levels in serum and CSF of patient and controls

		Patient	Controls (n=200)
Serum	Lactate[a]	1280-1700	600-1200
	Pyruvate[a]	118-126	75-120
	Ratio lactate/pyruvate	10.2-14.4	up to 15
CSF	Lactate[a]	1520-1830	1200-1600
	Pyruvate[a]	90-153	85-132
	Ratio lactate/pyruvate	12.0-16.9	up to 15

[a] μmol/l.

HISTOPATHOLOGIC STUDIES

Biopsies were taken from quadriceps muscle, sural nerve and liver. Histochemical and ultrastructural studies of quadriceps muscle showed no abnormalities. No 'ragged-red fibers' were noted in the trichromic stained section. The mitochondria were normal and no intramitochondrial inclusions were seen. On light and electron microscopic studies of sural nerve and liver no abnormalities were noted.

BIOCHEMICAL STUDIES

MATERIALS AND METHODS

Biochemical studies were performed in homogenized liver tissue obtained by needle biopsy, and two quadriceps muscle homogenates from biopsies obtained with a three-year interval.

Pyruvate oxidation rate in liver tissue homogenate was studied by measuring $^{14}CO_2$ production from [1-^{14}C]pyruvate according to Willems et al (1979). Pyruvate carboxylase activity was measured in liver homogenate as described by Utter and Keech (1963), with a regenerating system for acetyl-CoA according to Henning and Seubert (1964). Pyruvate oxidation rate and activity of citric acid cycle and respiratory chain were evaluated for muscle tissue by measuring $^{14}CO_2$ production from [1-^{14}C]pyruvate, [1-^{14}C]2-oxoglutarate and [U-^{14}C]malate in a 600 g supernatant, as described by Bookelman et al (1978b). Succinate-cytochrome *c* oxidoreductase activity was estimated in SETH medium (Bookelman et al, 1978b) at 25°C in the presence of 10 mM succinate, 2 mM potassium cyanide, 2 μg/ml rotenone and 50 μM cytochrome *c* as the final electron acceptor. The extinction was followed at 550 nm. An extinction coefficient of 21.1 $mM^{-1}.cm^{-1}$ was used (Van Gelder and Slater, 1962). Cytochrome *c* oxidase activity in muscle homogenate and isolated muscle mitochondria was measured according

to Cooperstein and Lazarow (1951). Cytochromes were assayed in isolated muscle mitochondria according to Bookelman et al (1978a). All oxidation rates and cytochrome determinations were performed in fresh tissue, the single enzyme measurements in samples stored at -70°C. Protein was assayed as described by Lowry et al (1951). The method of Parvin and Pande (1977) was used for determination of carnitine content in muscle homogenate.

RESULTS

In liver homogenate, [1-^{14}C]pyruvate oxidation was clearly decreased (Table 2), while pyruvate carboxylase had a normal activity (29 nmol/min/mg protein; control range 13 - 40, n=16). In muscle homogenate, the oxidation of [1-^{14}C]pyruvate, [U-^{14}C]malate and [1-^{14}C]2-oxoglutarate was markedly lowered under all incubation conditions tested (Table 2). These oxidation rates were not normalized in the presence of 25 μM cytochrome *c*, 1 mM NAD^+ and 0.1 mM CoASH, added alone or in combination. Furthermore, normal values were found for the cytochrome levels and cytochrome *c*

Table 2. $^{14}CO_2$ production from various ^{14}C-labeled substrates in liver and muscle tissue homogenates of patient and controls

	Substrate	Patient	Controls	No
Liver	[1-^{14}C]pyruvate + malate	19	45-205	13
Muscle[a]	[1-^{14}C]pyruvate + malate	80, 152[b]	243-729	41
	[1-^{14}C]pyruvate + carnitine	136, 164[b]	221-838	39
	[1-^{14}C]pyruvate + malate + malonate	76	214-687	36
	[U-^{14}C]malate + pyruvate + malonate	112, 189[b]	390-1172	39
	[U-^{14}C]malate + acetylcarnitine + malonate	126	352-1155	39
	[U-^{14}C]malate + acetylcarnitine + arsenite	75	172-492	36
	[1-^{14}C]2-oxoglutarate	86	341-1229	15

[a] nmol $^{14}CO_2$/h/mg protein, [b] determined in two specimens from biopsies obtained with a 3-year interval

Table 3. Contents of cytochrome *b*, *c* + c_1 and aa_3, and activities of cytochrome *c* oxidase and succinate-cytochrome *c* oxidoreductase in isolated muscle mitochondria of patient and controls

	Patient	Controls	No.
Cytochrome *b*[a]	329	228-424	14
Cytochrome *c* + c_1[a]	516	365-923	14
Cytochrome aa_3[a]	543	271-543	14
Cytochrome *c* oxidase[b]	2.17	1 29-3 43	19
Succinate-cytochrome *c* oxidoreductase[c]	110	75-186	7

[a] pmol/mg protein, [b] U/mg protein; [c] nmol succinate metabolized/min/g wet weight.

oxidase activity in isolated muscle mitochondria (Table 3). The activity of cytochrome *c* oxidase was also normal in liver and muscle homogenate. Muscle showed a normal activity of succinate-cytochrome *c* oxidoreductase (Table 3). Carnitine content of muscle was normal.

DISCUSSION

We reported on an eight-year-old girl with a mild spastic paraparesis, ataxia, exercise intolerance, and temporary deteriorations on intercurrent infections. Two sibs with similar clinical features died in the course of an intercurrent infection. One of them showed changes characteristic of SNE on neuropathologic examination. Considering the autosomal recessive mode of inheritance of SNE (Pincus, 1972) and the similarities in clinical picture of the three sibs, we concluded to the diagnosis of familial SNE.

In our patient, lactate and pyruvate levels of serum and CSF showed an intermittent elevation, a finding that suggested a disturbance in pyruvate metabolism. Biochemical studies in liver and muscle tissue confirmed the existence of a defective pyruvate catabolism.

In muscle homogenate, a decreased $^{14}CO_2$ production rate from [1-^{14}C]pyruvate either in the presence of malate or carnitine as an acetyl-CoA acceptor was found, indicating that the defect is not localized in the citric acid cycle. The decreased $^{14}CO_2$ production rate obtained with [U-^{14}C]malate in the presence of acetylcarnitine as acetyl-CoA donor

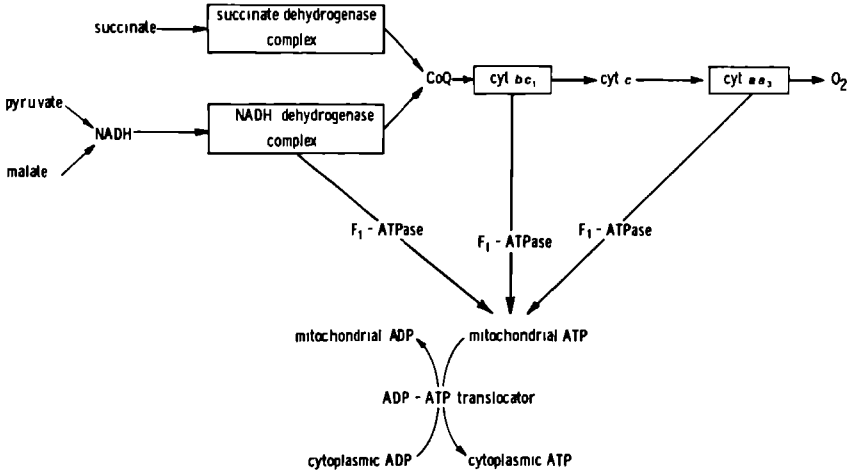

Fig 2. Simplified scheme of the mitochondrial respiratory chain, and synthesis and transport of ATP.

indicates that the defect is not localized in the pyruvate dehydrogenase complex (PDHc). The findings summarized in Table 2 can only be explained in terms of a defect in the mitochondrial electron transport chain, ATP-ADP translocator or F_1-ATPase (Fig 2). The afore mentioned conclusion is based on a previous observation that in human muscle homogenate malate and carnitine are equally effective as an acetyl-CoA acceptor, and pyruvate and acetylcarnitine as an acetyl-CoA donor (Bookelman et al, 1978b,c). As examination of the cytochrome content and succinate-cytochrome *c* oxidoreductase activity did not reveal abnormalities, only the first functional unit of the respiratory chain may be defective: the NADH dehydrogenase (Fig 2). Theoretically, lack of NAD^+ or CoASH could also cause low oxidative capacity of the mitochondria. We could exclude leakage of these cofactors out of the mitochondria as a cause of the defect because exogenous NAD^+ plus CoASH did not normalize oxidation rates.

Several authors have pointed to the association of SNE with disorders of oxidative metabolism. Since the initial publication of a pyruvate carboxylase (PC) deficiency in association with Leigh syndrome (Hommes et al, 1968), others reported the same finding (Grover et al, 1972; Tang et al, 1972; Gruskin et al, 1973; Gröbe et al, 1975; Maesaka et al, 1976; Van Biervliet et al, 1980; Gilbert et al, 1983). Only a few reports, however, link PC deficiency with pathologically proven SNE (Hommes et al, 1968; Tang et al, 1972; Van Biervliet et al, 1980; Gilbert et al, 1983), and even in these cases methods and interpretation of the findings remain controversial (Atkin et al, 1979; Murphy, 1981). A review of the literature on PC deficiency (Baal et al, 1981; DeVivo and Uziel, 1983) shows that (severe) PC deficiency can occur without evidence of SNE.

Deficiencies of pyruvate decarboxylase (Farmer et al, 1973; Blass et al, 1976; Hansen et al, 1982; Ohtake et al, 1982; Toshima et al, 1982) and defective activation of PDHc (DeVivo et al, 1979; Sorbi and Blass, 1982) have been reported in patients with SNE. The clinical manifestations of PDHc deficiency correlate wih the degree of impairment of activity (Blass, 1979). The paucity of correlative studies between deficient activity of PDHc and corresponding pathology makes it difficult to define the place of SNE in the spectrum of PDHc deficiency.

Defects of the respiratory chain have also been reported in Leigh syndrome. In 1977, Willems et al described the absence of cytochrome aa_3 and of cytochrome *c* oxidase activity in muscle tissue of a patient with SNE. Recently, Miyabayashi et al (1983) reported two siblings with cytochrome *c* oxidase deficiency, one of whom died of SNE. They demonstrated deficiency of cytochrome *c* oxidase activity in fibroblasts, liver, muscle and brain. Deficiencies of cytochrome *c* oxidase activity are also associated with the syndromes of fatal infantile mitochondrial my-

opathy with renal dysfunction (Van Biervliet et al, 1977), benign infantile mitochondrial myopathy (DiMauro et al, 1983), trichopoliodystrophy (Menkes' disease) (French et al, 1972), and progressive poliodystrophy (Alpers' disease) (Prick et al, 1983; Gabreëls et al, 1984).

In 1969, Pincus et al discovered an *in vitro* inhibition of the mitochondrial enzyme thiamine pyrophosphate-adenosine triphosphate phosphotransferase by urine and blood of a patient with SNE. The absence of thiamine triphosphate (TTP) in the brain of their patient led them to hypothesize that SNE was caused by this 'inhibiting factor'. Low values of TTP in SNE brains, roughly correlating with the sites of the brain lesions, were reported by the same author (Pincus et al, 1976). The hope that the urine inhibitor test would provide an unequivocal antemortem diagnosis of SNE has not been fulfilled. The urine test proved to have a 6% false-positive rate and in six out of twenty-nine patients no inhibiting factor could be demonstrated (Pincus et al, 1974).

There are several reports on NADH-Coenzyme Q oxidoreductase complex deficiencies (Morgan-Hughes et al, 1979; Busch et al, 1981; Land et al, 1981a,b; Prick et al, 1981; Morgan-Hughes, 1982). In contrast to the patients described by Morgan-Hughes et al (1979), Land et al (1981a) and Prick et al (1981), our patient shows no histochemical or ultrastructural abnormalities in muscle tissue.

The presence of a mitochondrial myopathy in combination with an encephalopathy in our patient makes her fit into the concept of mitochondrial encephalomyopathies (Shapira et al, 1977). Defects in oxidative metabolism in SNE, however, have also been shown in fibroblasts, liver and kidney. So, SNE is more than an encephalomyopathy: it is a multisystem disorder affecting derivatives of all three germinal layers. The disorder is associated with a disturbance in energy metabolism localized in the mitochondria.

Le Coultre and Ebels (1976) first used the term mitochondriopathy in a case of Canavan's disease. Walter (1981) proposed the concept of mitochondriopathy and he distinguished between a primary and a secondary form. On the analogy of this concept, SNE is a mitochondriopathy, manifesting as a distinct encephalopathy with well-characterized neuropathologic criteria and a defect of oxidative metabolism.

The recognition of distinct syndromes certainly has heuristic value, but the ultimate classification must depend on identification of etiology and pathophysiology of the individual disorders.

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CHAPTER IX

A MITOCHONDRIAL ENCEPHALOMYOPATHY WITH A PARTIAL CYTOCHROME *c* OXIDASE DEFICIENCY OF MUSCLE

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ABSTRACT

A-16-year-old girl shows delayed psychomotor development. In the infantile period, exercise intolerance, cerebellar signs, deteriorations on intercurrent infections, and disturbances of breathing and cardiac rhythm become manifest. From age 7 there is a chronic progressive psychomotor deterioration. Hypotonia, a bilateral pyramidal and cerebellar syndrome, and a mild epilepsy develop. Pyruvate and lactate levels are elevated in CSF, and lactate content is elevated in urine. There is an abnormally high rise of lactate levels on moderate exercise and an abnormal response to pyruvate loading. Quadriceps muscle biopsies obtained at age 10 and 16 years show ragged-red fibres, and a decreased cytochrome *c* oxidase activity and cytochrome aa_3 content. Cytochrome *c* oxidase activity in fibroblasts is normal. Clinical signs and symptoms in association with a disturbance of mitochondrial energy metabolism led us to a diagnosis of most probable Leigh syndrome.

INTRODUCTION

In the clinical spectrum of mitochondrial myopathies Petty et al[1] identified three subgroups: 1) chronic progressive external ophthalmoplegia (CPEO) and limb weakness, 2) proximal weakness with fatigability, 3) predominantly or exclusively central nervous system (CNS) disease. The recognition that CNS disease is a prominent feature in a subgroup of the mitochondrial myopathies prompted Shapira et al[2] to expand the concept of mitochondrial myopathy to mitochondrial encephalomyopathy. This latter group of disorders encompasses the syndromes of Alpers,[3] Leigh,[4] dysmyelination,[5] myoclonus epilepsy associated with 'ragged-red fibres' (MERRF)[6] and a syndrome indicated by the acronym MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes).[7]

We present a girl with a mitochondrial encephalomyopathy and a clinical diagnosis of most probable Leigh syndrome and a partial cytochrome *c* oxidase deficiency of muscle.

CASE REPORT

Our patient is the youngest child of healthy non-consanguineous parents. There is one healthy sib The oldest sib died at age 12 after an accident. Family history reveals no neurodegenerative disorders Two sibs of the father are suffering from epilepsy Pre-, peri- and postnatal periods of the index patient are uneventful. Psychomotor development is delayed She is able to sit without help at age 18 months and walk at age 3 years. She speaks her first words at age 2½ years. Motor performance is poor and deteriorations with often slow recovery, are noted on intercurrent infections During these periods of deterioration, frequent sighing, tachy- and bradypnea, and tachy- and bradycardia are evident. A few times she manifests a partial oculomotor nerve paresis on the left, that subsides within one week. A progressive exercise intolerance becomes evident At age 5 years, neurological examination reveals myopathic and cerebellar signs, active tendon reflexes and flexor plantar responses. Total IQ (WISC-R) is 95. At age 7 years, a slowly progressive psychomotor deterioration sets in, and absence-like epileptic manifestations appear, that are treated with ethosuccimide.

At age 10 years, she is referred to our department On examination we see a mentally retarded girl, with normal height, weight and skull circumference There are no degenerative stigmata General physical examination is normal. On neurological examination there is a generalized hypotonia, muscle wasting, and proximal muscular weakness There is an increased lumbar lordosis, recurvation of the knees and a positive Gowers' sign. Facial expression is poor with a mild bilateral ptosis, and speech is monotonous and dysarthric. Cerebellar involvement is evident by ataxia of rump and extremities, and marked dysdiadochokinesis. Tendon reflexes are hyperactive with spontaneous ankle clonus and bilateral Babinski signs

A steady slowly progressive deterioration of the neurological status is seen in the following years She is 16 years old now and total IQ is 53. She has been free of seizures and without anti-epileptic medication for the last 4 years

ELECTROPHYSIOLOGICAL AND RADIOLOGICAL INVESTIGATIONS

Electroencephalogram shows focal (right temporal) and generalized epileptic discharges. Electromyography and nerve conduction velocities are normal. Somatosensory, brainstem auditory and visual evoked potentials are within the normal ranges. Radiograms of skull, chest, hand and vertebral column, cerebral computed tomography scan and cerebral nuclear magnetic resonance scan are normal. Electrocardiogram is normal and the echocardiogram shows no signs of cardiomyopathy.

LABORATORY INVESTIGATIONS

Routine haematological parameters, serum electrolytes, serum pH, pCO_2 and bicarbonate, serum protein, protein- and immunoelectrophoresis, ammonia, lipids, short-chain fatty acids, lipoproteins and carnitine (total and free) are normal. Other studies reveal no abnormalities of renal, thyroid, parathyroid and adrenal functions. Serum enzymes, creatine kinase, serum levels of vitamins B_1, B_6, B_{12} and folic acid, serum levels of zinc, manganese, copper and ceruloplasmin, phytanic acid, uric acid, and activities of lysosomal enzymes in leucocytes are all normal. There are no acanthocytes. Syphilis, tuberculosis, sarcoidosis and systemic lupus erythematosus are ruled out by appropriate studies. Neurotropic virus antibody titers are not elevated in serum and cerebrospinal fluid (CSF). Urinalysis, urinary organic acids and amino acids, and urinary excretion of heavy metals are normal. Serum levels of glucose, pyruvate, lactate, β-hydroxybutyrate and acetoacetate, their ratios, and responses of glucose, pyruvate and lactate to oral glucose loading (1.75 g/kg) are in the normal ranges. Lactate excretion is elevated in one 24-hour urine sample (131 pmol/mmol creatine, normal <100). The McArdle/Fischbein ischaemic exercise test[8] yields a normal rise of serum lactate and ammonia levels. Moderate exercise (walking for a few minutes) gives a rise of serum pyruvate to 335 μmol/l (normal 60-155 μmol/l under resting conditions) and of serum lactate to 6480 μmol/l (normal 460-1720 μmol/l under resting conditions), the lactate-pyruvate ratio is 18.2 (normal up to 15) and serum pH remains normal with a base excess of -3.9. An intravenous pyruvate loading test[9,10] (500 mg/kg) shows an abnormal response in that there is a rise of the lactate level after 5 minutes postinfusion to a maximum at 15 minutes.

CSF cell number, protein content, protein- and immunoelectrophoresis, and the levels of glucose, β-hydroxybutyrate and acetoacetate are normal. The levels of pyruvate (222 μmol/l, normal 85-132 μmol/l) and lactate

(1900 μmol/l, normal 1200-1600 μmol/l) are elevated with a normal lactate-pyruvate ratio.

HISTOPATHOLOGICAL STUDIES

Quadriceps muscle biopsies are performed at the age of 10 and 16 years. Histopathological studies show no abnormalities except for the occurrence of ragged-red fibres in both biopsies. The cytochrome *c* oxidase (COX) stain shows a normal aspect in most fibres, but the ragged-red fibres show a strongly increased activity (Fig 1). Electronmicroscopy reveals no structural abnormalities of the mitochondria, and crystal-like inclusions do not occur. COX activity is normal in all fibres investigated (Fig 2).

BIOCHEMICAL STUDIES

MATERIALS AND METHODS

In fresh homogenate of quadriceps muscle obtained by biopsy at age 10

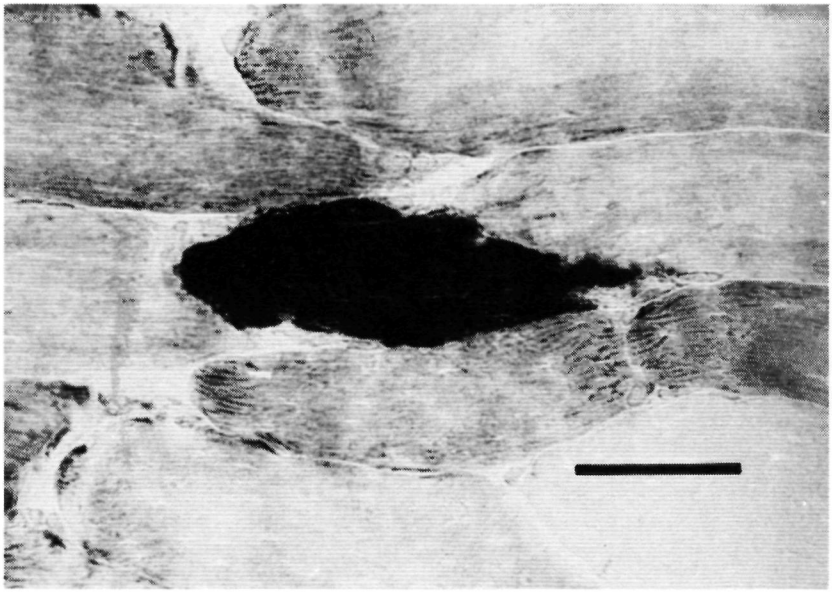

Fig 1. Cytochrome *c* oxidase stain. The fibres show a normal activity except for the ragged-red fibre in the middle, that shows a strong increase of activity (bar = 50 *μm*).

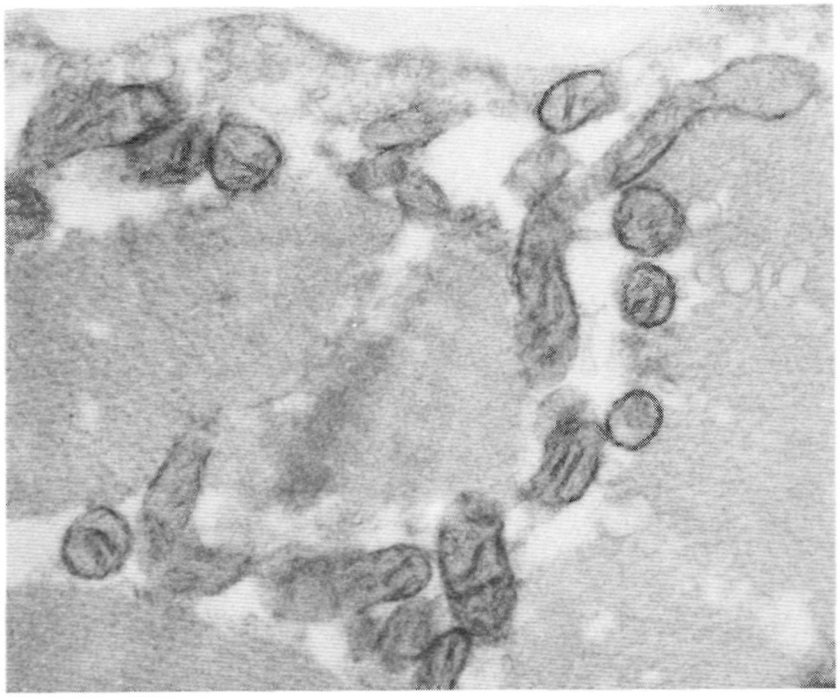

Fig 2. Electronmicrograph of section stained for cytochrome *c* oxidase activity. Normal reactivity is seen in all mitochondria (x60,000).

years, pyruvate oxidation rate and activities of citric acid cycle and respiratory chain are evaluated by measuring $^{14}CO_2$ production from [1-^{14}C]pyruvate and [U-^{14}C]malate.[11] ATP production by muscle homogenate is measured according to Ruitenbeek et al.[12] The activity of COX[13] is measured in muscle homogenate and in isolated mitochondria. Cytochrome content is measured in isolated mitochondria.[14] Protein is assayed according to Lowry et al.[15]

At age 16 years a quadriceps muscle specimen is obtained by needle biopsy. Carnitine content,[16] and activities of COX,[13] citrate synthase,[17] succinate-cytochrome *c* oxidoreductase[18] and NADH:O_2 oxidoreductase[19] are measured, after storage of the muscle at -70°C.

Oxidative metabolism is also evaluated in cultured fibroblasts by measuring $^{14}CO_2$ production rate from [1-^{14}C]pyruvate and [2-^{14}C]pyruvate,[20] and the activities of COX[12] and citrate synthase.[17]

RESULTS

Table 1 contains the biochemical findings in both muscle specimens. $^{14}CO_2$ production rates from [1-^{14}C]pyruvate and [U-^{14}C]malate by muscle homogenate are slightly diminished. ATP + CrP production with pyruvate as substrate is also decreased. The ratio of the ATP + CrP production to the arsenite sensitive pyruvate oxidation is normal. Total carnitine content (carnitine + acylcarnitines) is normal in muscle homogenate. The specific activity of COX in muscle homogenate and in isolated mitochondria is about 20% of the mean control value.This enzymatic defect is associated with a lack of cytochrome aa_3. The ratio of the content of this protein of the COX complex to the content of cytochrome b and $c + c_1$ is diminished (0.59 : 1 : 1.20; control ratio 1.08 : 1 : 1.52).

$^{14}CO_2$ production rates from [1-^{14}C]pyruvate and [2-^{14}C]pyruvate by fibroblasts, and the activities of COX and citrate synthase are normal.

DISCUSSION

We describe a 16-year-old girl with a slowly progressive degenerative neurological disorder characterized by myopathy, mental retardation, pyramidal, cerebellar and ocular signs. Frequent sighing, marked fluctu-

Table 1. Results of biochemical studies in the 600 g supernatant of the two muscle tissue specimens

	Patient		Controls		
	1980	1986	Range	Mean ± SD	No.
Oxidation					
[1-^{14}C]pyruvate + malate	208[1]		273-705	473 ± 117	20
[1-^{14}C]pyruvate + carnitine	237[1]		266-941	504 ± 169	20
[U-^{14}C]malate + pyruvate + malonate	270[1]		320-996	621 ± 211	20
[U-^{14}C]malate + acetylcarnitine + malonate	343[1]		317-1155	575 ± 220	20
[U-^{14}C]malate + acetylcarnitine + arsenite	196[1]		198-274	294 ± 80	19
ATP Metabolism					
ATP + CrP production from pyruvate	2075[2]		3354-9993	5910 ± 2168	20
ATP + CrP production / pyruvate oxidation	10.0		8 8-15 0	11.8 ± 1.8	20
Enzyme activities					
Citrate synthase		41[3]	48-146	77 ± 33	18
Cytochrome *c* oxidase	36[3]	41	73-284	194 ± 92	39
Succinate cytochrome *c* oxidoreductase		8.7[4]	10-33	18 ± 7	9
NADH O_2 oxidoreductase		15.3[5]	4 5-23	13 ± 7	7

[1] nmol $^{14}CO_2$/hr/mg protein, [2] nmol/hr/mg protein, [3] mU/mg protein, [4] nmol cytochrome *c* reduced/min/mg protein, [5] nmol NADH oxidized/min/mg protein

ations of respiratory and cardiac rate, and long-lasting deteriorations on intercurrent infections are other clinical characteristics.

Laboratory investigations show an elevation of lactate excretion in 24-hour urine. Lactate concentration shows an abnormal increase on moderate exercise, and after pyruvate loading. In CSF, levels of pyruvate and lactate are elevated. These findings are suggestive of a defect in pyruvate metabolism in this patient.

At the age of 10 and 16 years quadriceps muscle biopsies are performed for histochemical and biochemical studies. Histochemical studies show ragged-red fibres. Biochemical studies show a slight decrease of $^{14}CO_2$ production from pyruvate and malate, and a decrease of ATP production from pyruvate to one-third of the mean control value. COX activity in muscle homogenate as well as in isolated muscle mitochondria is decreased to 20% of the mean control value. These data and the lack of cytochrome aa_3 protein point to a disturbance of mitochondrial oxidative metabolism at the level of COX. Involvement of the central nervous system (CNS) and elevation of pyruvate and lactate in CSF make it likely that the same defect is present in CNS. Although the disorder has a multisystem character, no defect could be detected in fibroblasts.

In various patients with partial or complete COX deficiency, an overall decrease[21–23] or complete lack[24,25] of histochemical reactivity of COX have been reported. Apparently the residual activity of COX in our patient is sufficient for a normal light- and electronmicroscopic appearance.

COX deficiency clinically presents in a heterogeneous fashion. COX deficiency of muscle can manifest as a fatal infantile myopathy[26] with[27–34] or without[24,35–38] DeToni-Fanconi-Debré syndrome or as a benign spontaneously remitting myopathy of infancy.[21,39] COX deficiency has also been reported in two types of mitochondrial encephalomyopathies: the syndromes of Alpers[40] and Leigh.[22,25,39,41,42] Complete lack of histochemically demonstrable COX activity was reported in individual fibres in patients with chronic progressive ophthalmoplegia, but biochemical studies most often revealed normal COX activity.[43,44] The deficiency of COX described in Menkes' kinky hair disease[45] has a secondary nature, due to secondary deficiencies of copper dependent enzymes, one of which is COX.

Our patient fits in with the clinical picture of Leigh syndrome[46] (Table 2) and we have made a clinical diagnosis of Leigh syndrome in our patient.

Recently, a patient with a childhood encephalomyopathy with COX deficiency in muscle and platelets, resembling our patient, was reported by Angelini et al.[23] This patient, an 8-year-old boy, had muscle wasting with proximal weakness, ataxia and mental impairment, but no pyramidal signs. There was parental consanguinity in this case, pointing to an autosomal recessive mode of inheritance.

In other patients with Leigh syndrome, the deficiency of COX was present

Table 2. Clinical data of our patient compared with results of a literature study of 173 Leigh syndrome patients

	Patient	Literature (%)
Ocular signs	+	78
Respiratory signs and symptoms	+	69
Hypotonia	+	69
Pyramidal signs	+	61
Exercise intolerance	+	47
Cerebellar signs	+	39
Decompensation on infection	+	39
Mental retardation/deterioration	+	37
Seizures	+	36
Cardiac signs and symptoms	+	18

in skeletal muscle, heart muscle, brain, kidney, liver and cultured fibroblasts.[47] Normal enzyme activities have been reported in liver tissue,[25] and in liver tissue and cultured fibroblasts[47] of Leigh patients with a cytochrome *c* oxidase deficiency of muscle.

There are strong indications for the existence of tissue-specific isoforms of the complex enzyme COX,[48] that might explain the differential involvement of tissues. The apoprotein consists of multiple subunits, 3 of which are encoded by mitochondrial DNA, whereas the rest is encoded by nuclear DNA.[49] As mitochondrial DNA is maternally transmitted,[50] a non-mendelian type of inheritance is possible in cases of COX deficiency.[51] However, this has until now not been documented in respiratory chain defects, and autosomal recessive inheritance seems most probable.

To elucidate etiologic mechanisms, enzyme abnormalities will have to be clarified at the molecular level with modern techniques of immunocytochemistry and molecular genetics. Immunodetection allows identification of the enzyme subunit composition. The amount of immunologically reacting protein was normal in two Leigh patients with COX deficiency[41,52] and diminished[38] or absent[52] in patients with a fatal myopathy with or without renal dysfunction. As human mitochondrial DNA has been fully sequenced,[53] it is likely that mutations can be demonstrated at DNA level within a few years. Definition of biochemical errors at the molecular level will disclose different molecular defects of COX that constitute the basis of the different phenotypic expressions of the enzyme deficiency.

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CHAPTER X

FAMILIAL LEIGH'S SYNDROME

Association with a Defect in Oxidative Metabolism Probably Restricted to Brain

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ABSTRACT

Four siblings with Leigh's syndrome are described. The diagnosis was confirmed by pathological examination in one case. Chemical and biochemical investigations of serum and urine revealed no abnormalities of pyruvate metabolism, but all patients had marked elevations of CSF pyruvate and lactate concentrations. In three of the siblings, [1-^{14}C]pyruvate oxidation rates were normal in fibroblasts and leucocytes. In one patient, extensive biochemical and histochemical studies of liver and muscle tissue revealed no mitochondrial dysfunction. A defect of oxidative metabolism restricted to brain seems probable.

INTRODUCTION

The diagnosis of subacute necrotizing encephalomyelopathy or Leigh's syndrome rests on pathological findings. Sharply demarcated areas of spongy necrosis with capillary and glial proliferation and relative sparing of the neurons, localized in the grey matter of mid- and hindbrain, constitute the most prominent histological features (16). Clinical signs and symptoms show considerable variation and are non-specific (16,24). The mode of inheritance is autosomal recessive (16,24). Biochemical investigations have revealed associations with several defects of oxidative metabolism (11,15,37,40).

In our cases of familial Leigh's syndrome a defect of oxidative metabolism was probably restricted to the brain.

CASE REPORTS

The family consists of two healthy, unrelated parents and their four children, all suffering from a degenerative neurological disorder (Fig 1).

Case 1

Apart from slow psychomotor development, the patient's history was uneventful At age 6 years, his mental development appeared to be normal Movements were clumsy. There was pes cavus The tendon reflexes were hypoactive, Achilles tendon reflexes were absent. In the following years, his gait became ataxic and he often fell An action tremor of the upper extremities developed The legs became hypertonic with bilateral Babinski signs Pin-prick and vibration sense of the legs diminished With intercurrent infections there was clinical

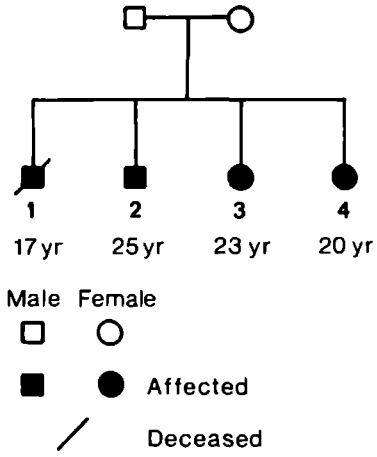

Fig 1. Pedigree of the family.

deterioration from which he took months to recover fully During such phases, the patient showed periodic bradycardia and tachycardia and tachypnoea Electroencephalography (EEG) showed a generalized slowing of background activity, and nerve conduction velocity studies and electromyography (EMG) showed a slight axonal polyneuropathy of the legs.

When aged 17 years, he died suddenly of respiratory arrest in the course of an intercurrent infection, during which he developed a tachycardia of 150 beats/min.

Pathological examination. The brain weighed 1,370 g and showed no macroscopic abnormalities On microscopical examination, sharply demarcated areas of spongy necrosis were situated symmetrically in the tegmentum of the brain-stem (from the caudal mesencephalon to the facial nerve nuclei) They were characterized by astroglia proliferation and capillary dilatation and proliferation with relative neuronal sparing. Identical lesions were found bilaterally in the thalamus. The medulla oblongata showed no abnormalities, except for capillary dilatation in the nuclei dorsales vagi In the cerebellum, there was glial proliferation in the dentate nuclei. The spinal cord showed marked myelin loss in the medial dorsal columns where the fibres originate from the lower extremities. On the basis of these pathological findings the diagnosis of Leigh's syndrome was made.

Case 2

After a normal psychomotor development, this patient went through a 3-month period of fever of unknown origin at age 3 years In this period, walking difficulties developed and gait deteriorated until he could no longer walk, owing to a severe spastic paraparesis with hyperactive tendon reflexes and Babinski signs. Recovery was slow and it took 1 year to reach the pre-morbid psychomotor level, but a rapid fatigability on exercise remained.

At age 6 years, during an attack of tonsillitis, he became apathetic, mute and ataxic. Hyperventilation and respiratory alkalosis developed. The respiratory rate normalized in a few months, but after that time periodic irregular breathing was seen. Recovery was only partial Motor retardation became evident. There was a choreoathetosis and dysdiadochokinesis. Muscle tone and reflexes were normal; plantar responses remained extensor. With intercurrent infections, there was always temporary deterioration

At age 10 years, a cerebellar dysarthria with pronounced dysdiadochokinesis was noted. Gait became more ataxic Muscle tone was low and reflexes were hypoactive with Babinski signs.

At age 15 years, there was a hypotonic paraparesis with absent tendon reflexes of the legs, extensor plantar responses and severe impairment of gait. Periods of hypothermia (32°-34°C) occurred.

The patient is now 24 years old. Full scale IQ is 70. He cannot walk without help. There is generalized muscular atrophy. He has a hypotonic tetraparesis with total areflexia, and ataxia of trunk and extremities, cerebellar dysarthria, hypermetria and intention tremor. Sensation is diminished distally in the extremities EEG shows diffuse encephalopathy with predominance of delta and theta activities Nerve conduction velocity studies and EMG show an axonal polyneuropathy Cerebral computed tomography (CT) scanning and cerebral angiography are normal.

Case 3

Psychomotor development was normal and medical history was uneventful until, at age 9 years, after an intercurrent infection, the patient insidiously developed walking difficulty and clumsiness of fine movements.

At age 13 years, a paraparesis and difficult micturition developed in the course of an intercurrent infection After initial improvement, a few months later, following a rubella inoculation, a bilateral oculomotor nuclear paresis became manifest. Respiration became irregular, there were bilateral pyramidal and cerebellar signs A few weeks later, her condition deteriorated rapidly A bulbar paresis developed into a total flaccid paralysis in 2 weeks. Artificial respiration was necessary for a month A sural nerve biopsy specimen showed active Wallerian degeneration of myelinated and unmyelinated fibres, with massive loss of

fibres. Recovery was only partial Since this time, the patient has been confined to a wheelchair. In the following years, there have been several periods of deterioration during metabolic stress. Periodic bradycardia and tachycardia were noticed with tachypnoea.

The patient is now 23 years old and functions on a moderately retarded mental level Respiration is spontaneous There is total incontinence. Vision is unimpaired despite bilateral optical atrophy There is divergence of the left eye, and eye movements are jerky with loss of smooth pursuit. There is a pseudobulbar paresis and a hypotonic paralysis of the legs. The arms have some residual motor function, which is further impaired by intention myoclonus and ataxia. Tendon reflexes are negative with Babinski plantar responses. Sensation is diminished distally in the legs. EEG shows a progressive diffuse encephalopathy EMG and nerve conduction velocity studies show an axonal polyneuropathy Cerebral CT scan is normal

Case 4

From age 4 years on, this patient insidiously developed neurological signs and symptoms, starting with easy fatigability and impairment of fine movement. Through the years there has been a slow, but steady progression, with stepwise deterioration during intercurrent infections In this patient, periods of tachypnoea, bradycardia and tachycardia, and hypothermia were also present.

The patient is now 20 years old and functions on a normal mental level She can manage the activities of daily life independently of others Outdoors, she is confined to a wheelchair. Speech shows a cerebellar dysarthria There is hypotonic tetraparesis with absent tendon reflexes and extensor plantar responses Ataxia of trunk and extremities, and an intention tremor indicate cerebellar involvement Sensation is unimpaired. EEG shows a diffuse encephalopathy EMG and cerebral CT scan are normal.

LABORATORY INVESTIGATIONS

Routine haematological parameters, serum electrolytes, serum pH, pCO_2 and bicarbonate, serum protein, NH_3, lipids and lipoproteins were normal. There were no acanthocytes. Other studies revealed normal liver, renal, thyroid, parathyroid and adrenal function tests, serum enzymes, serum levels of vitamin B_1, B_6, B_{12} and folic acid, serum copper and ceruloplasmin, phytanic acid, uric acid, amino acids, and lysosomal enzyme activities in leucocytes. Syphilis, tuberculosis, sarcoidosis and lupus erythematosus were ruled out by appropriate studies. Neurological virus antibody titres and toxoplasma antibody titres were normal in serum and cerebrospinal fluid (CSF). Urinalysis, urinary organic acids and amino acids, and urinary excretion of heavy metals were normal. Serum glucose, pyruvate and lactate, and responses of glucose, pyruvate and lactate to oral glucose loading (1.75 g/kg) and 24-h urinary lactate excretion were all in the normal ranges. In case 2, prolonged fasting for 24 h resulted in a normal rise in serum lactate and pyruvate, and a normal stimulation of ketogenesis (17). Also in case 2, the intravenous alanine loading test (500 mg/kg) (12) yielded normal results. The results of the intravenous pyruvate loading test (8), however, were abnormal (Figs 2,3).

CSF cell count was normal in all patients. In cases 1 and 2, CSF protein content was elevated: 580 and 436 mg/l respectively (normal <350 mg/

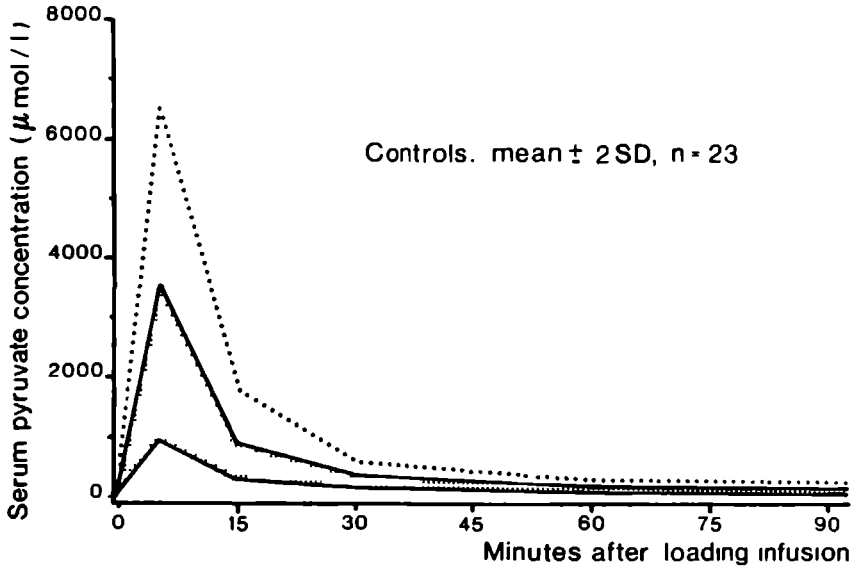

Fig 2. Patient 2: pyruvate response to pyruvate loading (500 mg sodium pyruvatye/kg body weight is administered in a period of 10 min using a 0.91 M sodium pyruvate solution (8)).

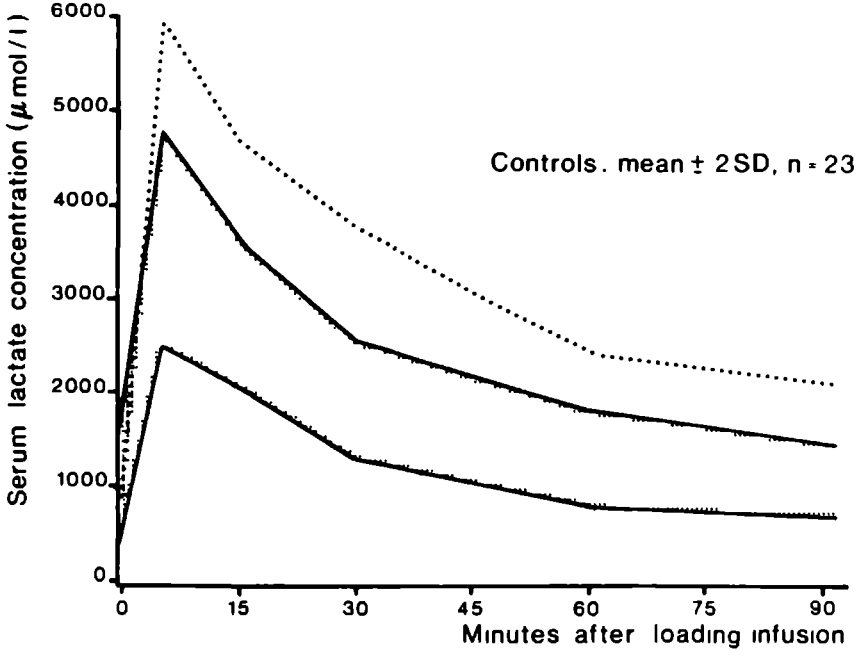

Fig 3. Patient 2. lactate response to pyruvate loading (for details see legend to Fig 2).

l). Protein electrophoresis and immunoelectrophoresis patterns of all patients were normal, as were the levels of glucose and minerals. CSF pyruvate and lactate levels were elevated in all patients; the lactate/pyruvate ratio was elevated in cases 2 and 4 (Table 1).

BIOPSY STUDIES

In case 2, biopsies were performed on quadriceps muscle and liver at age 24 years. Histochemical studies were performed in muscle tissue. Biochemical studies were performed on liver and muscle homogenates.

MORPHOLOGICAL STUDIES

Histochemical studies of quadriceps muscle showed the presence of atrophic angulated fibres, indicative of denervation. There were no signs of lipid myopathy. In the trichromic stained section, no 'ragged-red' fibres were seen. No ultrastructural studies were performed.

BIOCHEMICAL STUDIES

Pyruvate carboxylase activity was measured in liver tissue homogenate (35). The activities of the enzymes cytochrome *c* oxidase, citrate synthase, succinate-cytochrome *c* oxidoreductase and carnitine palmitoyltransferase (at 0.8 mM palmitoylcarnitine) were measured in liver and/or muscle tissue (4,13,29,32). Pyruvate oxidation rate, and activities of citric acid cycle and respiratory chain enzymes were evaluated in muscle homogenate by measuring $^{14}CO_2$ production from [1-^{14}C]pyruvate and [U-^{14}C]malate (2). ATP metabolism was investigated after inhibition of adenylate kinase activity (28). Carnitine content was measured in muscle homogenate (23). Protein was assayed according to the method of Lowry et al (18).

In cases 2-4, pyruvate oxidation and citric acid cycle activities in leucocytes and fibroblasts were determined by measuring $^{14}CO_2$ production from [1-^{14}C]pyruvate and [2-^{14}C]pyruvate (41).

Table 1. CSF levels of lactate and pyruvate, and the ratio lactate/pyruvate in all four cases

	Case 1	Case 2	Case 3	Case 4	Controls (n=200)
Lactate[a]	4125	3760	2440	4250	1200-1600
Pyruvate[a]	225	201	200	211	85-132
Ratio L/P	9.9	18.7	12.2	20.1	up to 15

[a] μmol/l.

Table 2. Activities of mitochondrial enzymes[a] in liver and muscle tissue of case 2

Substrate	Case 2	Controls		
		Range	Mean ± SD	No
Liver				
Citrate synthase	7 6	11-46	27.4 ± 14 3	12
Cytochrome *c* oxidase	33	28-60	41 8 ± 10 9	7
Pyruvate carboxylase	9 1	13-40	26 1 ± 8 4	16
Muscle				
Citrate synthase	11 2	27-77	44 7 ± 15 0	18
Cytochrome *c* oxidase	83	73-284	194 ± 92	39
Succinate-cytochrome *c* oxidoreductase	8 2	11-35	18 ± 7	14
Carnitine palmitoyltransferase	0 25	0 42-0 97	0 62 ± 0 15	14

[a] mU/mg protein

Table 3. $^{14}CO_2$ production[a] from [1-^{14}C]pyruvate and [U-^{14}C]malate in muscle tissue homogenates of case 2 and controls

Substrate	Patient	Controls (n=19)	
		Range	Mean ± SD
[1-^{14}C]pyruvate + malate	2 04	1 89-8 74	3 53 ± 1 73
[1-^{14}C]pyruvate + carnitine	2 75	1 89-9 60	3 68 ± 1 91
[U-^{14}C]malate + pyruvate + malonate	2 20	1 81-10 12	4 69 ± 2 41
[U-^{14}C]malate + acetylcarnitine + malonate	2 24	1 61-8 69	4 29 ± 2 25
[U-^{14}C]malate + acetylcarnitine +arsenite	1 27	1 06-4 63	2 20 ± 1 08

[a] nmol/hr/mU cytochrome *c* oxidase

RESULTS.

Activities of the mitochondrial enzymes measured in liver and muscle tissue were all in the low-normal range (Table 2). Studies of [1-^{14}C]pyruvate and [U-^{14}C]malate oxidation by intact muscle mitochondria revealed normal oxidation rates (Table 3). The ratio of the number of moles ATP + CrP produced per mole pyruvate oxidized amounted to 8.7 (control range: 8.8 - 15.0; n=20). Carnitine content (total and non-esterified) was normal in muscle tissue. [1-^{14}C]pyruvate and [2-^{14}C]pyruvate oxidation rates in leucocytes and fibroblasts (cases 2-4) were all within the normal ranges.

DISCUSSION

We have described a family in which all of four sibs are afflicted with a degenerative neurological disorder. The oldest sib (case 1) died at age 17 years in the course of an intercurrent infection, and on pathological

examination the typical features of subacute necrotizing encephalomyelopathy or Leigh's syndrome were found. Leigh's syndrome is transmitted as an autosomal recessive trait (19) and familial occurrence is not rare (16,24). Therefore, in our opinion the diagnosis can be considered established in the other sibs. A mitochondrial type of inheritance (9) would explain better the occurrence of the disorder in all four of the children, but the mother is healthy and her family history gives no evidence of myopathic or degenerative neurological disease. The signs and symptoms of our patients and the chronic progressive course with exacerbations, mostly on intercurrent infections, have been described in Leigh's syndrome (6).

Histochemical studies of muscle tissue revealed no abnormalities, in particular no signs of mitochondrial myopathy, e.g. ragged-red fibres or lipid myopathy. Ragged-red fibres, however, have only rarely been reported in Leigh's syndrome (5). Ultrastructural studies were not performed.

Laboratory investigations revealed a marked elevation of CSF pyruvate and lactate levels with normal serum levels in all patients.

Biochemical studies in liver and muscle tissue of case 2 revealed moderately low activities of all mitochondrial enzymes (Table 2). This finding indicates a low mitochondrial content in both tissues and not a specific enzyme deficiency. This is in accordance with the normal amount of pyruvate and malate that can be oxidized by muscle mitochondria, as well as the normal amount of ATP produced from the substrates (Table 3). Although the oxidative capacity may be somewhat limited per gram of muscle, it is obviously not so seriously that it results in lactate accumulation in the blood. Direct measurement of pyruvate carboxylase in liver tissue demonstrated a normal activity when compared with the other mitochondrial enzymes. A pyruvate dehydrogenase complex (PDHc) deficiency can be excluded in the present patient as [1-^{14}C]pyruvate is oxidized at a normal rate. Normal $^{14}CO_2$ production by [1-^{14}C]pyruvate is only possible if there is normal PDHc activity.

Mitochondrial function being normal in muscle, liver, leucocytes and fibroblasts, it seems most probable that there is a disturbance of pyruvate metabolism, as indicated by the marked elevation of CSF pyruvate and lactate levels, which is restricted to the brain. The elevated levels of pyruvate and lactate after intravenous pyruvate loading can be explained by long-term immobilization, generalized muscular atrophy, limitation of oxidative capacity of muscle (as discussed above) and decrease of cerebral pyruvate oxidation. A cerebral biopsy for exact localization of the site of the biochemical defect was not performed.

Several biochemical disorders of mitochondrial function have been described in Leigh's syndrome. Reports of associations with pyruvate carboxylase deficiency (14,15,33,36) and a disturbance of the thiamine

triphosphate synthesis system (25, 26) have been controversial. There have been reports of subacute necrotizing encephalomyelopathy associated with cytochrome *c* oxidase deficiency (20,40). Recently, a tissue specificity of cytochrome *c* oxidase was demonstrated that might explain the differential involvement of tissues (e.g. the central nervous system in our patients) in cytochrome *c* oxidase deficiency (21,42). A deficiency of NADH dehydrogenase was reported in two patients (37,38). The most consistent biochemical finding in Leigh's syndrome, however, seems to be an abnormality of PDHc. Decreased PDHc activities (1,10,11,22,34) and defective PDHc activation (3,7,31) have been reported. In a patient with Alpers' syndrome a deficiency of pyruvate dehydrogenase restricted to the brain has been demonstrated (27). In a recent report, however, Sheu and Blass (30) were unable to demonstrate a significant difference in the activity of pyruvate dehydrogenase phosphate phosphatase, the enzyme that activates PDHc by phosphorylation, in cultured skin fibroblasts of five patients with Leigh's syndrome compared with normal control cell lines.

In view of a biochemical disorder of mitochondrial function in Leigh's syndrome, the concept of mitochondriopathy (as proposed by Walter (39)) seems appropriate in subacute necrotizing encephalomyelopathy. However, the pathophysiology of Leigh's syndrome, especially the regional selective vulnerability, i.e. the predilection for specific brain structures in the expression of a biochemical defect, is still unknown.

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CHAPTER XI

GENERAL DISCUSSION

This study provides a review of the literature data concerning Leigh syndrome, adding the results of own clinical, neurophysiological, and biochemical investigations in patients with this syndrome. It provides data and arguments to answer the questions raised in the first chapter:

1. Can a better characterization of the clinical picture of Leigh syndrome be achieved?
2. Is it possible to distinguish clinically distinct subgroups within the spectrum of Leigh syndrome?
3. What is the contribution of technical (neurophysiological, neuroradiological and biochemical) investigations to diagnosis and to insight into the pathophysiology of Leigh syndrome?
4. Is it possible to formulate better criteria for a diagnosis of Leigh syndrome durante vitam?

Pathophysiological and therapeutic aspects and future perspectives will also be discussed in this chapter.

RESULTS AND ANSWERS TO THE QUESTIONS

Can a better characterization of the clinical picture of Leigh syndrome be achieved?

The median age at onset of the disease in the 173 patients from the literature is 0.9 years, and the median age at death is 2.1 years. In the juvenile group, median time of survival after onset of the disease is 5.5 years versus 0.9 years in the neonatal, early infantile, and infantile groups. The male : female ratio is 3 : 2, but with increasing age at onset there is an increasing preponderance of males over females, up to 4 : 1 in the juvenile group, which is significantly deviant from the expected 1 : 1 ratio. We have no explanation for this phenomenon. The mode of inheritance of Leigh syndrome is generally considered to be autosomal recessive, but an X-linked mode of transmission might explain the male preponderance.

Signs and symptoms, both at onset and in the course of the disease (the latter for different age groups) are presented in Chapter II. Feeding problems, motor retardation, hypotonia, eye signs and symptoms, mental retardation, seizures, and respiratory problems are seen most frequently at onset (Table 3, Chapter II). Eye signs and symptoms, respiratory problems, hypotonia, pyramidal signs, motor retardation, feeding problems, exercise intolerance, deterioration on infections, cerebellar signs, mental retardation, seizures, extrapyramidal and cardiac signs are frequently encountered in the course of the disease (Table 4, Chapter II).

Respiratory and cardiac signs and symptoms, pyramidal and cerebellar

signs, and especially the decompensation/deterioration during infectious episodes have been noticed more frequently in our patient group than in the patients reported in the literature (Table I, Chapter IV). It is evident that it is not one separate sign or symptom but the combination of signs and symptoms that may point to Leigh syndrome.

Is it possible to distinguish clinically distinct subgroups within the spectrum of Leigh syndrome?

For practical purposes the patients reported in the literature have been divided into 4 subgroups according to age at onset, although these subgroups appear to represent no separate clinical entities. The group with the juvenile age at onset can be separated from the rest, in that the disease runs a much more protracted course in these children and median survival is much longer than in the other groups (Table 2, Chapter II). The patients with a very rapidly progressive course of the disease clinically present in a rather uniform way with epileptic seizures and acute coma, ending in death within a few days. The more subacute and protracted the disease, the more manifold the clinical signs and symptoms. From the case studies reported in the literature we were not able to separate any subgroup with a characteristic clinical presentation. But in our patient group we found 3 patients with a very similar clinical picture consisting of hypokinesia and rigidity (Chapter III). The importance of this finding is, that in the differential diagnosis of infantile and juvenile parkinsonism Leigh syndrome must be taken into account, and that hypokinesia and rigidity in a child warrant investigation of pyruvate metabolism.

What is the contribution of technical investigations to diagnosis and to insight into the pathophysiology of Leigh syndrome?

Neurophysiological investigations

The value of neurophysiological studies in Leigh syndrome is assessed in 12 patients (Chapter IV). As neurophysiological studies (electroencephalogram, electromyogram, nerve conduction velocity studies and evoked potentials) rarely show abnormalities specific for a certain disease, it is not surprising to find no specific abnormalities in our patients. But the yield of neurophysiological abnormalities in our patients is surprisingly low and especially the lack of evoked potential abnormalities is striking (Table 4, Chapter IV). In our patients there is a relatively late onset and a chronic, slowly progressive course of the disease; clinical examination

points to pathology not of the afferent nervous system structures but especially of the efferent structures. This might explain the paucity of neurophysiological abnormalities in the patients we studied.

Radiological investigations

A radiological abnormality in Leigh syndrome becoming increasingly recognized is the finding of bilateral hypodense areas in the basal ganglia, especially the putamina, on CT or MRI. Two of our patients show these hypodensities (Chapter III), whereas 4 other proven Leigh patients do not show the abnormality. So these lesions on CT or MRI are not obligatory, nor pathognomic of Leigh syndrome, as they are found in other pathological conditions (Chapter III). However, if these lesions are present the possibility of Leigh syndrome should be considered. An interesting finding in MRI investigation of our patients was the fact that the calculated T_1 values of the thalamus of two patients were prolonged while the images were not abnormal. It might, therefore, be useful to calculate the T_1 and T_2 values of clinically relevant areas, even if MRI pictures are not abnormal, in patients suspected of Leigh syndrome.

Biochemical investigations

Leigh syndrome is associated with several defects of pyruvate metabolism and of the respiratory chain (Chapter II). Chemical studies of pyruvate metabolism in our patients (determination of pyruvate and lactate levels in serum and cerebrospinal fluid (CSF), of lactate in a 24-hour urinary sample, and of glucose, pyruvate, and lactate after oral glucose loading) often point to a disturbance of pyruvate metabolism (Table 2, Chapter V). In the literature, data on chemical studies of pyruvate metabolism in Leigh patients are relatively scarce. But in the patients in whom lactate and pyruvate are determined in body fluids abnormalities are often found (Table 5, Chapter II). The most important abnormal parameters in our patients are the levels of pyruvate and lactate in CSF, whereas the pyruvate and lactate levels in serum are only rarely elevated in resting state (Table 2, Chapter V). The intravenous pyruvate loading test, performed in 9 patients with Leigh syndrome and 23 control subjects, proved to be a valuable diagnostic tool to detect disturbances in pyruvate metabolism (Chapter V). By constructing a tolerance region for normal controls we made the test suitable for diagnostic purposes (Fig 1, Chapter V). The intravenous pyruvate loading test gives no additional information when routine investigations indicate a disturbance of pyruvate metabolism; but when these investigations are inconclusive, while at the same time there is a strong clinical suspicion of Leigh syndrome, the intravenous pyruvate loading test can be of diagnostic value.

The case studies of our own patients with Leigh syndrome reveal interesting facts. A defect of NADH dehydrogenase was established in two patients (Chapters VI and VII) and suspected in one patient (Chapter VIII). This biochemical defect was hitherto only reported in patients with muscular complaints and in a patient with Alpers' disease (Chapter VI). In one patient we found a defect of cytochrome *c* oxidase (Chapter IX). The association between Leigh syndrome and cytochrome *c* oxidase deficiency has been described by several authors, but cytochrome *c* oxidase deficiency has also been associated with several other heterogeneous clinical syndromes with variable biochemical expression in different organs (Chapter IX). The last case study describes four sibs with Leigh syndrome (Chapter X). No abnormalities of pyruvate metabolism or of the respiratory chain were found in liver and muscle tissue, but the marked elevation of pyruvate and lactate levels in CSF points to a disturbance of pyruvate metabolism restricted to brain.

Is it possible to formulate better criteria for a diagnosis of Leigh syndrome durante vitam?

Despite the lack of characteristic signs and symptoms, and despite the existence of some overlap, the clinical picture of Leigh syndrome differs clearly from those of the other mitochondrial encephalomyopathies. It is, in our opinion, possible to come to the diagnosis of Leigh syndrome durante vitam when the following conditions are present:

- clinical signs and symptoms,
- bilateral hypodense areas in the basal ganglia region on CT or MRI,
- association with a defect of pyruvate metabolism or respiratory chain,
- autosomal recessive mode of inheritance.

SOME PATHOPHYSIOLOGICAL CONSIDERATIONS

The pathophysiology of Leigh syndrome so far has not been clarified. Radiological studies (CT, MRI) and chemical studies can have important contributions to the diagnosis, but both techniques provide little insight into the pathophysiology.

Morphological mitochondrial abnormalities have been frequently observed in patients with defects of the respiratory chain or of the energy transducing system.[1] The structural mitochondrial alterations are not specific for any type of disorders, and no correlation has been found between type of morphological mitochondrial alteration and a certain enzyme deficiency.

In many patients with Leigh syndrome defects of pyruvate metabolism and the respiratory chain have been reported. Defects of the pyruvate dehydrogenase complex (PDHc) and cytochrome *c* oxidase (COX) may be etiopathogenic factors in Leigh syndrome.[2] A causal link of a pyruvate carboxylase deficiency with Leigh syndrome seems unlikely (Chapter VIII). In favour of a relationship between PDHc deficiency and Leigh syndrome is the pathological similarity between Leigh syndrome and Wernicke's encephalopathy, the latter being associated with a deficiency of thiamine (vitamin B_1), a cofactor of PDHc.

The striking clinical deterioration of Leigh patients in periods of infections, inoculations or traumas, all of which are events leading to an increased energy demand, is compatible with the concept of deficiencies of enzymes involved in oxidative metabolism. Without metabolic stress the residual enzyme activity is sufficient for normal mitochondrial or cellular functioning, but with increasing energy demands the system decompensates at its weakest link (the deficient enzyme) and clinical symptoms become manifest.

The fact that deficiencies of PDHc, NADH dehydrogenase and COX all affect the capacity of mitochondrial oxidative metabolism might explain the occurrence of one clinicopathological entity (Leigh syndrome), based on these different defects (Fig 3, Chapter II). But defects of PDHc, NADH dehydrogenase and COX have also been reported in association with several other clinical syndromes: PDHc deficiency in Alpers' disease, hypomyelination, and spinocerebellar ataxias; NADH dehydrogenase deficiency in patients with a clinical myopathy and with Alpers' disease; and COX deficiency in early infantile, rapidly progressive, fatal myopathy with and without renal dysfunction, a benign infantile myopathy, and Alpers' disease. According to Blass,[3] in PDHc deficiency the clinical picture seems to show a positive correlation with the degree of residual activity of PDHc, while in NADH dehydrogenase and COX deficiency such a correlation has not been demonstrated.

The mitochondrial encephalomyopathies are multisystem disorders. The biochemical abnormalities have been detected in various tissues, e.g. muscle, liver, brain, kidney and cultured fibroblasts. However, in most patients the defects are expressed only in skeletal muscle and brain tissue. Exceptionally, defects restricted to brain have been published[4] (the family described in Chapter X). The presence of a biochemical abnormality does not mean that there are always clinical correlates, e.g. a biochemical defect of muscle can be established in a patient who does not show clinical myopathy. In patients with an encephalomyelopathy there is often such a 'biochemical' mitochondrial myopathy.

Insight into the biochemical defect at a molecular level has to be gained to be able to explain the different phenotypic expressions of the enzyme

deficiencies. In COX deficiency the different tissue-specific isozymes of COX might explain the differential involvement of tissues, which may constitute one of the causes of the various clinical syndromes associated with deficiency of this enzyme.

THERAPY

Many therapeutic strategies have been reported in patients with disturbances of pyruvate metabolism or the respiratory chain (see Chapter II). As soon as the diagnostic investigations were completed, all our patients received a combination of thiamine (3 x 100 mg) and lipoic acid (3 x 20 mg), both cofactors of PDHc, and riboflavin (3 x 5 mg), a precursor of the flavin moiety of flavoproteins (see Chapter II). The results of this therapy have not been evaluated consistently by means of a clinical trial, nor by comparing results of chemical and biochemical studies before and during therapy.

In order to evaluate the efficacy of drug treatment regimens in this group of disorders, prospective controlled trials will have to be performed in patients with identified biochemical defects. As the incidence of these disorders is very low, a multicenter approach will be necessary.

ANTENATAL DIAGNOSIS

Of the various abnormalities found in the mitochondrial (encephalo)-myopathies only a restricted number of enzyme deficiencies can be demonstrated in fibroblasts.[5] The activities of cytochrome *c* oxidase and the pyruvate dehydrogenase complex can be determined in both chorionic villi and fibroblasts, and thus antenatal diagnosis of defects in these enzymes is possible, if the defects come to expression in these cells. Besides other advantages of the use of chorionic villi, the technical problems in determining the activities of the respiratory chain are fewer in the case of chorionic villi than in fibroblasts.

Robinson et al[6] claim that it is possible to detect deficiencies of NADH dehydrogenase and cytochrome *c* oxidase in cultured fibroblasts of all patients in whom chemical studies point to a deficiency of the respiratory chain. However, in patients with (encephalo)myopathies, in whom distinct enzymatic defects were established in muscle, we found no decreased enzyme activities in fibroblasts and chorionic villi. This limits, of course, the possibilities for antenatal diagnosis.

FUTURE PERSPECTIVES

Several recent and future developments will have their impact on the diagnosis of Leigh syndrome and the insight into its pathophysiology. The increasing resolution of CT and MRI will enable detection of the often very small lesions in the brainstem, that may constitute the only morphological abnormalities in Leigh syndrome.

A very promising new development is the *in vivo* phosphorus magnetic resonance spectroscopy (^{31}P-NMR) of muscle, that already permits the identification of a variety of metabolic disorders of muscle[7,8] and the monitoring of therapeutic improvements in mitochondrial (encephalo)-myopathies.[9] Rapid, non-invasive diagnosis of disturbances of mitochondrial functioning will probably result from the further development and application of ^{31}P-NMR spectroscopy.

Positron emission tomography (PET) has already enabled *in vivo* study of cerebral glucose metabolism in normal brain and in several neurological disorders in a quantitative way.[10] Improvement of spatial resolution (from 7-8 mm at present to 2-3 mm in the future), and reduction of processing time per event, so that rapid changes in (patho)physiological parameters can also be recorded with accurate assignment to small cerebral structures, will contribute to more insight in the pathophysiology of Leigh syndrome. The quantitative analysis of the metabolism of therapeutic substances labeled with positron-emitting radionuclides will permit *in vivo* monitoring of therapy. Perhaps PET will help to solve the problem of selective regional vulnerability of certain brain structures. ^{31}P-NMR spectroscopy and PET will enable the clinician to make the diagnosis in affected but still asymptomatic sibs of Leigh patients. Such technical possibilities will be of great value in genetic counseling.

Biochemical investigations in various tissues are being used to identify enzyme deficiencies of pyruvate metabolism or respiratory chain in Leigh syndrome and other mitochondrial (encephalo)myopathies. With use of modern techniques of immunocytochemistry and of molecular genetics attempts are made to disclose the abnormalities at the molecular level. Probably this will solve the problems of tissue and organ specificity of some enzyme deficiencies and of the different phenotypic expressions of certain enzyme deficiencies. Of course, also therapy will benefit by exact localization of the molecular defect.

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SUMMARY / SAMENVATTING

SUMMARY

This study of Leigh syndrome is focusing mainly on the clinical and biochemical aspects of the disorder.

In Chapter II the results of a literature study of 173 patients with proven Leigh syndrome are summarized. This group of patients is divided into four subgroups according to age at onset: neonatal (0-4 weeks), early infantile (4 weeks-1 year, infantile (1-4 years) and juvenile (4-16 years). Sex ratio, duration of the disease, and most important signs and symptoms at onset and in the course of the illness are surveyed. The results of technical studies (neurophysiological studies, radiological studies, and chemical and biochemical studies) and their significance are discussed. An overview of the pharmacotherapeutical regimens and strategies in this group of disorders is presented. The main conclusion of this study is, that it is possible to come to a diagnosis of 'most probable' Leigh syndrome durante vitam based on 1) signs and symptoms, 2) autosomal recessive mode of inheritance, 3) association with a defect of pyruvate metabolism or respiratory chain, and 4) bilateral hypodense areas in the basal ganglia region on CT or MRI.

In Chapter III three patients are reported in whom hypokinesia and rigidity are the most prominent clinical manifestations of Leigh syndrome. The importance of investigating mitochondrial energy metabolism in children with parkinsonism is stressed.

In Chapter IV the results of neurophysiological studies (electroencephalogram, electromyogram, nerve conduction velocity studies, visual evoked potentials, brainstem auditory evoked potentials, and median nerve somatosensory evoked potentials) in 12 patients are presented. No specific abnormalities are found and it is concluded that neurophysiological studies at least in our patients - a subgroup with a relatively late onset and a chronic progressive course of the disease - do not contribute to the diagnosis of Leigh syndrome.

Chapter V contains the results and implications of an intravenous pyruvate loading test in 9 patients with Leigh syndrome. The pyruvate loading test can have diagnostic value in cases where there is a strong clinical suspicion while determinations of lactate and pyruvate levels in body fluids are not conclusive.

In Chapters VI, VII and VIII case studies are presented of two patients with an established (Chapters VI and VII) and one patient with a suspected (Chapter VIII) NADH dehydrogenase deficiency. Such a defect of the respiratory chain was hitherto not reported in Leigh syndrome.

In Chapter IX a patient is described with a clinical diagnosis of Leigh syndrome and a cytochrome *c* oxidase deficiency in muscle. This defect,

which is associated with several clinical syndromes, has been reported in a number of Leigh patients.

Chapter X contains a report of four siblings with Leigh syndrome associated with a probable defect in oxidative metabolism restricted to brain.

SAMENVATTING

Deze studie betreffende het syndroom van Leigh is voornamelijk gericht op de klinische en biochemische aspecten van de aandoening.

In Hoofdstuk II worden de resultaten weergegeven van een literatuurstudie van 173 patiënten waarbij het syndroom van Leigh is vastgesteld. Deze groep patiënten is verdeeld in vier subgroepen naar leeftijd bij het begin van de ziekte: neonataal (0-4 weken), vroeg-infantiel (4 weken-1 jaar), infantiel (1-4 jaar) en juveniel (4-16 jaar). Er wordt een overzicht gegeven van de sex-ratio, de duur van de periode tussen vaststellen van de ziekte en het overlijden, en de belangrijkste klinische verschijnselen bij het begin en in het verloop van de ziekte. De resultaten en de betekenis van technische onderzoeken (neurofysiologisch, radiologisch, chemisch en biochemisch onderzoek) worden gepresenteerd en besproken. Er wordt een overzicht gegeven van de farmakotherapeutische mogelijkheden bij deze groep van aandoeningen. De voornaamste conclusie van dit onderzoek is dat het mogelijk is om durante vitam tot een diagnose van 'zeer waarschijnlijk' syndroom van Leigh te komen op basis van: 1) klinisch beeld, 2) autosomaal recessieve erfelijkheidsmodus, 3) stoornis in het pyruvaatmetabolisme of in de ademhalingsketen, en 4) bilaterale afwijkingen in de basale ganglia op CT of MRI.

In Hoofdstuk III worden 3 patiënten besproken waarbij hypokinesie en rigiditeit de belangrijkste klinische manifestaties van het syndroom van Leigh vormen. Het belang van het onderzoek van het mitochondriële energiemetabolisme bij kinderen met parkinsonisme wordt benadrukt.

In Hoofdstuk IV worden de resultaten weergegeven van neurofysiologisch onderzoek (elektroencefalografie, elektromyografie, bepaling van de geleidingssnelheid van zenuwen en evoked potentials) bij 12 patiënten uit de Nijmeegse kliniek met het syndroom van Leigh. Er worden geen specifieke afwijkingen gevonden. Geconcludeerd wordt dat het neurofysiologisch onderzoek in ieder geval bij onze patiënten - een subgroep met een relatief laat begin van de aandoening en een chronisch progressief beloop - geen bijdrage levert aan de diagnose.

In Hoofdstuk V worden de resultaten en implicaties besproken van een intraveneuze pyruvaatbelastingstest bij 9 patiënten met het syndroom van Leigh. Deze test is ontworpen om het pyruvaatmetabolisme te onderzoeken. De intraveneuze pyruvaatbelastingstest kan diagnostische waarde hebben in gevallen waar klinisch een sterke verdenking bestaat op het syndroom van Leigh, waarbij metingen van laktaat en pyruvaat in lichaamsvloeistoffen geen duidelijke indicatie geven omtrent het al of niet bestaan van een stoornis in het pyruvaatmetabolisme.

In Hoofdstukken VI, VII en VIII wordt de studie beschreven van twee patiënten (Hoofdstukken VI en VII) met een bewezen en van één patiënt

(Hoofdstuk VIII) met een waarschijnlijke deficiëntie van het NADH dehydrogenase. Dit defect van de ademhalingsketen was tot nu toe nog niet beschreven bij het syndroom van Leigh.

In Hoofdstuk IX wordt een patiënt beschreven met een klinische diagnose van het syndroom van Leigh en een cytochroom *c* oxidase deficiëntie van het spierweefsel. Dit defect, dat geassocieerd is met verschillende klinische syndromen, is beschreven bij een aantal patiënten met het syndroom van Leigh.

In Hoofdstuk X worden vier kinderen uit één gezin beschreven met het syndroom van Leigh, met waarschijnlijk een stoornis in het oxidatieve metabolisme welke beperkt is tot het centrale zenuwstelsel.

DANKWOORD

Dit onderzoek werd verricht vanuit de Afdeling Kinderneurologie* (hoofd Prof.Dr. F.J M. Gabreels) van het Instituut voor Neurologie (hoofd. Prof.Dr. B.P.M. Schulte) van het Sint Radboudziekenhuis te Nijmegen. De studie vormt een onderdeel van het research-programma van de Werkgroep Onderzoek Neuromusculaire Aandoeningen (voorzitter: Dr. J.M.F. Trijbels).

De medewerkers van de volgende instituten en afdelingen wil ik allen hartelijk danken voor hun bijdragen aan de totstandkoming van dit proefschrift:

- Het Instituut voor Neurologie, en wel in het bijzonder:
  - de Afdeling Kinderneurologie
  - de Afdeling Klinische Neurofysiologie (hoofd: Prof Dr. S.L.H. Notermans)
  - het Research Laboratorium voor Morfologische Neurologie (waarnemend hoofd· Dr H.J ter Laak)
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- Het Instituut voor Submicroscopische Morfologie (hoofd: Prof.Dr. A.M. Stadhouders)
- De Afdeling Neuroradiologie (hoofd: Prof.Dr H O.M. Thijssen) van het Instituut voor Radiodiagnostiek
- Het Instituut voor Neurochirurgie (hoofd: Prof.Dr. E. Meijer)
- De Werkgroep Erfelijkheidsadviezen (hoofd: Drs. B.C.J. Hamel) van het Instituut voor Anthropogenetica
- De Werkgroep Intermediair Metabolisme (hoofd: Prof Dr. J H. Veerkamp) van de Vakgroep Biochemie
- De Mathematisch-Statistische Adviesafdeling (waarnemend hoofd: Drs. W H Doesburg)
- De Medische Bibliotheek (hoofd Mevr.Drs. S. Bakker)

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* Medio 1986 geïntegreerd met de Afdeling Neuropediatrie van het Instituut voor Kindergeneeskunde (hoofd Prof Dr G B A Stoelinga) tot het Interdisciplinair Kinderneurologisch Centrum (hoofd Prof Dr F J M Gabreëls)

CURRICULUM VITAE

De schrijver dezes werd op 17 januari 1952 geboren te Eindhoven. Na in 1972 het eindexamen Gymnasium ß behaald te hebben aan het Van der Putt Lyceum te Eindhoven, studeerde hij van 1972 tot 1979 geneeskunde aan de Katholieke Universiteit te Nijmegen. Het kandidaatsexamen en het doctoraal examen werden cum laude behaald.

Van juli 1979 tot september 1984 volgde hij de opleiding tot het specialisme zenuw- en zielsziekten: de 3-jarige A-opleiding neurologie aan de Kliniek voor Neurologie van het St. Radboudziekenhuis te Nijmegen, opleiders Drs. J.J. Prick -ad interim- en Prof.Dr. B.P.M. Schulte, en de 2-jarige A-opleiding psychiatrie aan de Psychiatrische Instituten van de Godshuizen te 's Hertogenbosch, opleider Dr. G.J. Zwanikken.

Vervolgens werd de opleiding gevolgd ter verkrijging van de aantekening klinische neurofysiologie op de Afdeling Klinische Neurofysiologie van de Kliniek voor Neurologie van het St. Radboudziekenhuis, opleider Prof.Dr. S.L.H. Notermans.

Sinds 1 januari 1985 is hij werkzaam als neuroloog-klinisch neurofysioloog in het Ignatiusziekenhuis te Breda, in associatief verband met M.A.M. Bomhof, M.E. Dorren, W.A.C. Schijns (tot september 1985) en J.P.M. Stroy (vanaf september 1985).

STELLINGEN

behorende bij het proefschrift

LEIGH SYNDROME

in het openbaar te verdedigen
op 9 oktober 1987
des namiddags te 3.30 uur

door

P.M.M. VAN ERVEN

1. Op basis van klinische verschijnselen, bilaterale afwijkingen in de basale ganglia op cerebrale CT scan en MRI, associatie met een stoornis in het pyruvaatmetabolisme en een autosomaal recessieve erfmodus kan men, durante vitam, de diagnose 'zeer waarschijnlijk' syndroom van Leigh stellen.
   - dit proefschrift

2. Als de gehalten aan pyruvaat en laktaat in serum en liquor cerebrospinalis, de laktaatuitscheiding in 24-uurs urine en de resultaten van de orale glucosetolerantietest en van de pyruvaatbelastingstest normaal zijn, is de pyruvaatoxidatie van spier- en hersenweefsel ongestoord.
   - dit proefschrift

3. Klinisch-neurofysiologische onderzoeksmethoden dragen niet bij tot het stellen van de diagnose syndroom van Leigh.
   - dit proefschrift

4. Bij kinderen met een hypokinetisch-rigide syndroom moet de diagnose syndroom van Leigh overwogen worden en dient het pyruvaatmetabolisme onderzocht te worden.
   - dit proefschrift

5. Het hypokinetisch-rigide syndroom op kinderleeftijd wordt vaak niet herkend en men stelt dan ten onrechte meestal de diagnose spastische tetraplegie bij een infantiele encephalopathie.

6. De therapie van de (akute) hernia nuclei pulposi (HNP) bestaat uit absolute bedrust gecombineerd met analgetica en spierrelaxantia. Wertheim Salomonson stelde in 1911 reeds, hoewel de HNP in zijn tijd nog niet bekend was: "tegen massage bij versche gevallen der neuralgia ischiadica dient met nadruk te worden gewaarschuwd".
   - Wertheim Salomonson JKA. Amsterdam, Scheltema en Holkema's Boekhandel, 1911, p 315.

7. In de Chinese Volksrepubliek zijn de incidentie en prevalentie van cerebrovasculaire aandoeningen gelijk aan die in Westerse landen, doch om onbekende redenen is het aantal intracerebrale bloedingen (44%) veel groter dan in Westerse landen gemiddeld gezien wordt (12%).
   - Li S-c, et al. Neurology 1985;35:1708-13.

8. Bij patiënten met een asymptomatische carotissouffle is het geven van uitgebreide informatie omtrent mogelijke symptomen van voorbijgaande ischemische aanvallen van belang, want "although the risk of cerebral ischemic events is highest in patients with severe carotid artery stenosis, in most instances even these patients do not have strokes without some warning".
   - Chambers BR, et al. N Engl J Med 1986;315:860-5.

9. Huidafwijkingen (pruritus, pigmentafwijkingen en hyperkeratotische veranderingen) worden gezien bij ongeveer 30% van de patiënten met hersentumoren.
   - Andreev VC, Petkov I. Br J Dermatol 1975;92:675-8.

10. Bij onderzoek naar de nauwkeurigheid van citaten en referenties in medische tijdschriften (waaronder de Lancet, New England Journal of Medicine en British Medical Journal) bleek, dat 24% van alle referenties onjuist geciteerd werd. Vele onwaarheden worden op deze manier geaccepteerde feiten.
    - De Lacey G, et al. Br Med J 1985;1:557-9.

11. Het om budgettaire redenen onthouden van medische zorg aan bepaalde groepen patiënten door ziekenhuisdirekties of medici is ten principale onjuist. Het betreft hier geen medische doch een politieke keuze. Laat de politiek zich duidelijk uitspreken omtrent wie in de toekomst wèl en wie géén behandeling mag verwachten.

12. Door het grotendeels negeren van risicofaktoren m.b.t. het ontstaan van ziekten, geeft de Nederlandse bevolking impliciet aan vertrouwen te hebben in de beoefenaars van de geneeskunst hier te lande.